

# **For Reference**

---

**NOT TO BE TAKEN FROM THIS ROOM**

Ex LIBRIS  
UNIVERSITATIS  
ALBERTAENSIS











Thesis  
1377  
40D

THE UNIVERSITY OF ALBERTA  
MEASUREMENT OF VASODILATATION IN  
THE SUBMAXILLARY GLAND OF THE CAT



by  
Edward Karpinski

A THESIS  
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
OF DOCTOR OF PHILOSOPHY

DEPARTMENTS OF PHYSIOLOGY  
AND ELECTRICAL ENGINEERING

EDMONTON, ALBERTA

SPRING, 1971





Thesis,  
1971  
41D

UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Measurement of Vasodilatation in the Submaxillary Gland of the Cat" submitted by Edward Karpinski in partial fulfillment of the requirements for the degree of Doctor of Philosophy.



## ABSTRACT

A new flow meter has been designed and developed to measure the venous outflow from the submaxillary gland of the cat. The flow meter is of the thermal type and measures a wide range of venous flows. The sensing device is a thermistor which is kept at a constant temperature by feedback of electrical power and this change in power gives a measure of fluid velocity which is independent of thermistor characteristics. A second thermistor is used as a reference to provide corrections for temperature changes in the fluid and to provide a balance for zero velocity. The flow meter has been applied to the study of vasodilatation in the submaxillary gland of the cat. Its rapid response to changes in venous outflow on parasympathetic nerve stimulation enables the detection of vasodilatation in less than one half of a second after nerve stimulation. The latency between parasympathetic nerve stimulation and hyperpolarization of secretory cells is approximately the same as that of vasodilatation. This would support the direct innervation of the vasculature by nerve fibers rather than a metabolic vasodilator such as the kallikrein-kinin system.



## ACKNOWLEDGMENTS

I wish to express my appreciation for the encouragement and assistance that I received during the preparation of this thesis. This work was carried out under the supervision of Dr. M. Schachter to whom I wish to express my sincere appreciation for advice and assistance throughout the work.

I also wish to express my sincere appreciation to Professor Y.J. Kingma for his advice and assistance and for acting as a co-supervisor of this thesis.

I wish to express my appreciation to Drs. Barton, Newsham and Vaneldik for their assistance. I am also thankful to the staff and graduate students of the Department of Physiology for their help and advice and to my wife, Eileen, for her help and understanding during the preparation of this thesis.

For the assistance in the preparation of the figures, I am very thankful to Fred Loeffler and Ken Burt for their excellent work.

Financial support from the Alberta Heart Foundation and the Medical Research Council is gratefully acknowledged.



## Table of Contents

Chapter 1	MEASUREMENT OF BLOOD FLOW	1
1.1	Introduction	1
1.2	Electromagnetic Flow Meter	2
1.3	Ultrasonic Flow Meter	3
1.4	Drop Counter	4
1.5	Heated Thermocouple or Thermistor	4
Chapter 2	FORCED CONVECTION FLOW METER	5
2.1	Summary of Methods	5
2.2	General Specifications of Flow Meter	5
2.3	Thermistor	6
2.4	Self-Heated Thermistor	7
2.5	Probe	9
2.6	Flow Meter Scheme	11
2.7	Thermistor Biasing	14
Chapter 3	STEADY STATE RESPONSE OF FLOW METER	15
3.1	Introduction	15
3.2	Calibration of Probe	15
3.3	Theoretical Calculation of Heat Transfer	21
3.4	Temperature Compensation	28
Chapter 4	BANDWIDTH OF FLOW METER	31
4.1	Time Constant of the Thermistor	31





4.2	Bandwidth of System	34
4.3	Stability of the System	38
Chapter 5 CONTROL OF BLOOD FLOW TO THE SUBMAXILLARY GLAND		
		42
5.1	Literature Review	42
5.1A	Blood Flow and Secretion	42
5.1B	Electrophysiology	51
5.2	Objectives of Present Work	56
5.3	Methods	57
5.3A	Animals and Anaesthesia	57
5.3B	Nerve Stimulation	57
5.3C	Salivary Flow	57
5.3D	Blood Flow	58
5.3E	Electrophysiological Experiments	60
5.4	Results	62
5.4A	Electrophysiology	62
5.4B	Blood Flow	66
5.4C	Experiments with Bradykinin Potentiating Factor	73
5.5	Discussion	78
5.6	Conclusions	83
Bibliography		84
Appendix 1 ACHIEVED SPECIFICATIONS		94



Appendix 2	CIRCUITS	95
A2.1	General Scheme	95
A2.2	Heating Circuit	95
A2.2a	Oscillator	96
A2.2b	Schmitt Trigger and Flip Flop	96
A2.2c	Modulator	97
A2.2d	Resonant Amplifier	98
A2.2e	Power Stage	99
A2.3	Measuring Thermistor and Filter	100
A2.4	Compensating Thermistor	101
A2.5	Amplifier	102
A2.6	Linearizing Circuits	104
A2.6a	Square Root Circuit	104
A2.6b	Squaring Circuit	104
A2.7	Meter Circuit	107
A2.8	Power Supplies	107



## List of Figures

### Figure

- 1 Steady state voltage current characteristics of a Fenwal BC32L1 thermistor at fluid temperatures of 19.5 and 37° C. 8
- 2 Glass tube used for probe. 10
- 3 Approximate location of the two thermistors in the glass tube. 11
- 4 Basic scheme of flow meter 13
- 5 Initial flow meter scheme used for calibrations. 16
- 6 Calibration curve for 1.6 mm. diameter probe. 17
- 7 Calibration curve of 1.6 mm. diameter probe. 18
- 8 Complete scheme of the forced convection flow meter. 19
- 9 Calibration curves for the forced convection flow meter. 20
- 10 Comparison of theoretically calculated heat transfer from a constant temperature thermistor to the measured heat transfer. 25
- 11 A comparison of the calibration curve obtained in this work to the calibration curve reported by Grahn, Paul and Wessel. 27
- 12 Error in zero balance versus temperature deviations from 37° C. 30
- 13 Signal flow diagram of forced convection meter. 33



14	Response of flow meter to step air flow.	35
15	Response of system to a $\frac{1}{4}$ ohm change of resistance in series with the thermistor.	36
16	Root loci of the system.	41
17	Schematic diagram of the experimental set-up used in the blood flow experiments.	59
18	Schematic diagram of experimental set-up used in the electrophysiological experiments.	61
19	Secretory cell response to 5 stimuli of the chorda tympani nerve.	63
20	Secretory cell response to a single stimulus of the chorda tympani nerve.	63
21	Secretory cell response to sympathetic nerve stimulation.	65
22	Secretory cell response to sympathetic nerve stimulation.	65
23	Vasodilatation in the cat submaxillary gland as a result of chorda tympani nerve stimulation.	67
24	Vasoconstriction in the cat submaxillary gland as a result of sympathetic nerve stimulation.	68
25	Volume increase versus number of stimuli.	69
26	Vasodilatation in the cat submaxillary as a result of a 3 second period of chorda tympani nerve stimulation.	70
27	Time delay between pressure change in the sub- maxillary vein and the resultant flow change in the probe.	74





28	Vasodilatation in the submaxillary gland as a result of chorda tympani nerve stimulation before and after B.P.F.	75
29	Potentiating effect of B.P.F. on the bradykinin response of the guinea pig ileum.	77
A-1	Block diagram of flow meter.	95
A-2	Block diagram of heating circuit.	96
A-3	Oscillator.	97
A-4	Modulator.	98
A-5	Resonant amplifier.	99
A-6	Power stage.	100
A-7	Measuring thermistor and filter.	101
A-8	Compensating thermistor.	102
A-9	Amplifier.	103
A-10	Square root circuit.	105
A-11	Diode network.	106
A-12	Squaring circuit and range control.	106
A-13	Diode network.	107



## List of Tables

Table 1	Shortest latencies between chorda tympani nerve stimulation and onset of vasodilatation.	71
---------	--	----



## List of Symbols

$\alpha$	temperature coefficient of resistance in a thermistor.
B	magnetic field strength.
$\beta$	constant depending on thermistor material (4000).
$C_p$	specific heat of the fluid at constant pressure.
C	heat capacity of the thermistor
D	thermal diffusivity of the fluid in $\text{cm}^2/\text{sec}$ .
$\nabla$	vector operator.
$\delta$	boundary layer (velocity).
$e_o$	output voltage.
E	electric field strength.
$G_1 \text{---} G_n$	transfer functions.
H	transfer function.
$\kappa$	thermal conductivity of the fluid milliwatts/cm.
$K \text{---} K_n$	static gain constants.
$K(v)$	dissipation factor.
Nu	Nusselt number (dimensionless heat transfer coefficient).
$\theta$	spherical co-ordinate.
P	power in milliwatts.
$\rho$	density of the fluid in $\text{gm}/\text{cm}^3$ .
Pr	Prandtl number.
Re	Reynold's number.
$r_o$	radius of thermistor.
r	spherical co-ordinate.



$R$	resistance.
$R_0$	initial resistance.
$s$	Laplace transform.
$\Delta T$	temperature difference.
$T$	temperature.
$\tau \cdots \tau_n$	time constants.
$t$	time.
$\nu$	Kinematic viscosity.
$V$	velocity.





### 1.1 Introduction

Blood flow measurement techniques are numerous and are worthy of being called a highly developed science. This science has developed as an off-shoot from techniques developed by physicists and engineers used to measure the flow of fluids. The techniques for measuring blood flow are, of course, modifications due to the unique problems encountered when attempting to measure blood flow in humans and in undisturbed experimental animals. The main techniques in use are clearance methods, electromagnetic and ultrasonic flow meter methods. (The clearance methods are direct applications of the Fick principle which states that the blood flow through a gland or organ is the amount of substance cleared per unit of time divided by the arteriovenous concentration difference produced by this clearance.) Other methods are the heated thermocouple, or thermistor, and the drop counter. These latter methods are very sensitive but, with the exception of the drop counter, are difficult to calibrate. Another technique is plethysmography, which measures volume changes in a limb or an organ. It does not however separate the flows in the various tissues of the limb.

In physiology a problem may be the control of blood flow to an organ or gland, so that the knowledge of sudden changes in blood flow in one vessel is important. The



useful techniques in this case are then limited. One can not, for example, use a clearance technique since it would not show rapid changes. Plethysmography could be used but is usually very difficult to apply to a whole gland or organ, and if water is used as the surrounding fluid it would damp out any rapid changes in flow.

Other techniques which could be used employ the following:

- (1) electromagnetic flow meter
- (2) ultrasonic flow meter
- (3) drop counter
- (4) heated thermistor or thermocouple.

## 1.2 Electromagnetic Flow Meter

The correct name for this type of flow meter is the electromagnetic induction flow meter. The principle behind it is described by Faraday's Law which states that an electrical potential will be developed across a conductor (blood) moving through a magnetic field. The magnitude of the electrical potential induced is proportional to the magnetic field strength and the velocity of the moving conductor.

$$E \propto BV \qquad \dots (1.1)$$

where B = magnetic field strength and

V = velocity.

If the conductor is circular and the velocity profile



is axially symmetrical, the induced voltage is proportional to the average velocity.

In practice, the electromagnet is driven by either a sine wave or a square wave of current. This presents problems since the electrodes and wires leading from the electrodes form the secondary of a transformer; the primary being the driving coil of the electromagnet. This quadrature voltage may be larger than the flow induced voltage. Several methods are used which overcome this problem. The most popular is one in which the electromagnet is driven by a fairly high frequency square wave and the induced voltage is sampled when the rate of change of the applied field is zero.

### 1.3 Ultrasonic Flow Meter

The ultrasonic technique consists of two methods. One method is based on the physical phenomenon that the effective velocity of sound through a moving medium is the sum of the velocity of sound relative to the medium and the velocity of the medium. The other method which is much simpler to apply, is the Doppler Shift sonic flow meter method. When a sound wave is reflected from a moving target, the frequency of the reflected sound differs from the frequency of the incident sound by a quantity proportional to the velocity of the target relative to the sound source. This type of flow meter is easy to build and is accurate to a very low flow range, but it does not discrim-





inate the direction of flow.

#### 1.4 Drop Counter

The drop counter counts drops of blood via a light and photocell arrangement while a rate meter or recorder records the flow. It is useful for small flows but its disadvantages are speed of response, and the fact that some arrangement must be made to put the blood back into the animal. Also when the flow is high it cannot distinguish between drops.

#### 1.5 Heated Thermocouple or Thermistor

The heated thermocouple or thermistor principle is usually based on the fact that the elements are heated above the temperature of the blood. Blood flow past the heated element cools it by forced convection. Changes in temperature are detected by bridge arrangements and give indications of the flow rate. Another application is one in which the heated element detects the changes in thermal conductivity of the tissue as a result of changes in blood flow. These methods are usually slow because of the thermal time constants of the elements and, since they are non-linear, they are very difficult to calibrate. They are however, very sensitive and will measure very small velocities.





## 2.1 Summary of Methods

The problem of measuring flows to and from glands and organs is partially solved. For example, if the flow is greater than several cc./min., and is arterial, electromagnetic or ultrasonic techniques can be employed. If the flow is small and venous, the only linear instrument which can be used is the drop counter. However there are difficulties with its speed of response as it responds very slowly. In order to study the control of blood flow to the submaxillary gland of the cat, where basal flows are of the order of 0.25 to 1.0 cc. per minute and the arterial supply is very diffuse and inaccessible, a new technique for flow measurement had to be developed.

## 2.2 General Specifications of Flow Meter

Since high sensitivities are required, this indicates that a thermal technique would be the most plausible, for example, a heated thermocouple or thermistor. Thermal devices are very sensitive and are the most useful form of transducer capable of measuring the low velocities involved in venous outflows from glands. Since thermistors can be made very small, they are the logical choice for the sensor. Also, their thermal times are quite small. The bandwidth of a thermistor sensor, as is done in hot wire anemometry, may be increased by supplying feedback as has been attempted



by Katsura, Weiss, Baker and Rushmer (1959) and Grahn, Paul and Wessel (1968). The thermistor, in order to eliminate thermal delays and to improve the speed of response, must be in the flow stream whereas the probe, to compensate for changes in blood temperature, must have two thermistors--one which measures the flow and one which compensates for the minute temperature changes of the blood.

Several possibilities exist for the placement of the thermistor:

- (1) at the end of a flexible catheter
- (2) inside a needle
- (3) inside a rigid tube which, through a double cannulation, becomes part of the blood vessel.

For the particular application in mind (the venous outflow from the submaxillary gland) the latter method was chosen due to the size of the vessel and the location in the animal.

### 2.3 Thermistor

The detecting element of the flow meter is a thermistor which is a semi-conductor of ceramic material. It is made by sintering mixtures of metallic oxides such as manganese, nickel, cobalt, copper, iron and uranium. Thermistors are resistors with a high negative temperature coefficient of resistance. As the temperature increases, the resistance decreases. The mathematical expression which relates the



resistance and absolute temperature of the thermistor is as follows:

$$R_O(T) = R_O(T_O) e^{\beta \left( \frac{1}{T} - \frac{1}{T_O} \right)} \quad \dots (2.1)$$

where  $R_O(T)$  = the resistance at absolute temperature  $T$

$R_O(T_O)$  = the resistance at absolute temperature

$T_O$  and

$\beta$  = a constant which depends on the material used to make the thermistor.

Equation 2.1 applies to an indirectly heated thermistor; one in which no power is being dissipated.

## 2.4 Self-Heated Thermistor

If a very small voltage is applied to a thermistor, a small current will flow which does not produce enough heat in the thermistor to heat it measurably above its surroundings. Under these circumstances, Ohm's Law will be followed and the current will be proportional to the applied voltage. However, if the voltage is gradually increased, the current will increase and the heat generated in the thermistor will raise its temperature above that of its surroundings. The resistance will consequently be lowered and more current will flow. Figure 1 shows that the voltage drop across a thermistor (Fenwal BC32L1) increases as the current increases until it reaches a peak value beyond which the voltage drop decreases as the current further increases. In the latter portion of the curve the thermistor





is exhibiting a negative resistance characteristic. Figure 1 shows the characteristics of a BC32L1 thermistor at two fluid temperatures--19.5 and 37° C.

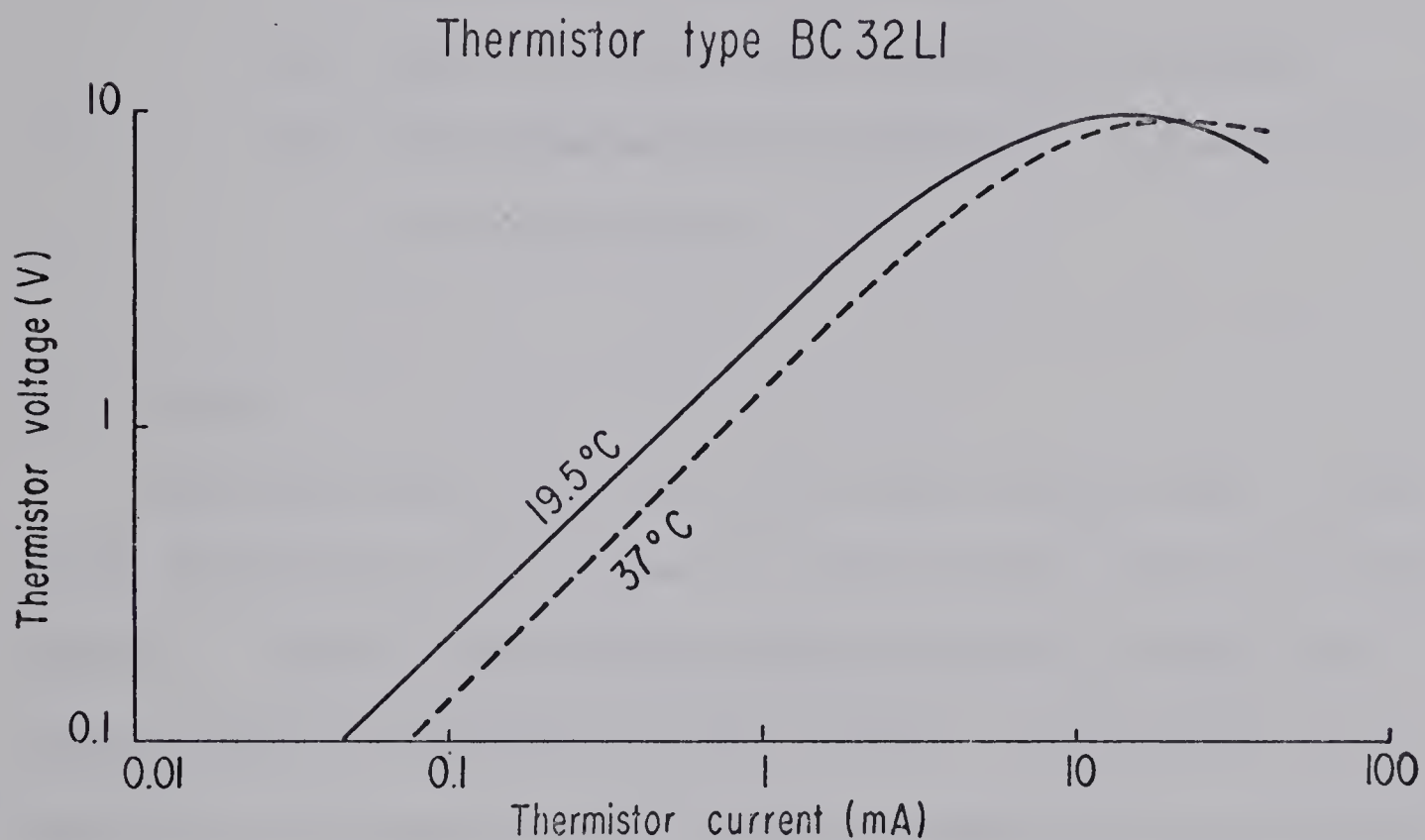


Figure 1. Steady state voltage current characteristics of a Fenwal BC32L1 thermistor at fluid temperatures of 19.5 and 37° C. The fluid is 0.16M saline.

The self-heated thermistor situated in a fluid is cooled by heat conduction, free and forced convection and radiation. It is generally accepted that radiation effects may be neglected if the temperature difference between the





fluid and thermistor is small (Hinze, 1959). Free convection effects are independent of fluid velocity. The total amount of heat transferred from a self-heated thermistor to the fluid due to forced convection depends upon:

- (1) flow velocity
- (2) the difference in temperature between the thermistor and the fluid
- (3) the physical properties of the fluid
- (4) the dimensions and physical properties of the thermistor.

## 2.5 Probe

The probe was made of a thin wall glass tube. The probe diameters were chosen to fit various sizes of blood vessels. Glass tubing was used so that the thermistor leads could be soldered to the tubing. This was done in order to overcome the difficulty encountered in attaching leads to the thermistor leads. The thermistor leads are 0.001 inch platinum iridium wire. The bead is approximately 0.007 inch with a specified resistance of 2 kilohms at 20° C. To construct the probe, holes were drilled in the glass tube at right angles 0.5 mm. apart and 0.25 mm. in diameter. Figure 2 shows the glass tube used in a typical probe.

Two thermistors were inserted into the probe and were staggered off center to prevent one thermistor from blocking the flow to the other one. The thermistor leads were



attached to the tubing by painting with conducting epoxy. To this epoxy long wire leads were soldered. The 0.25 mm. holes through which thermistor leads passed were completely plugged with silicone rubber and the long wire leads were then epoxied onto the glass with conventional epoxy to give a good mechanical contact. The epoxy was then coated with a thin layer of silicone rubber in order to make it resistant to moisture. Figure 3 shows the approximate location of the two thermistors in the glass tube.

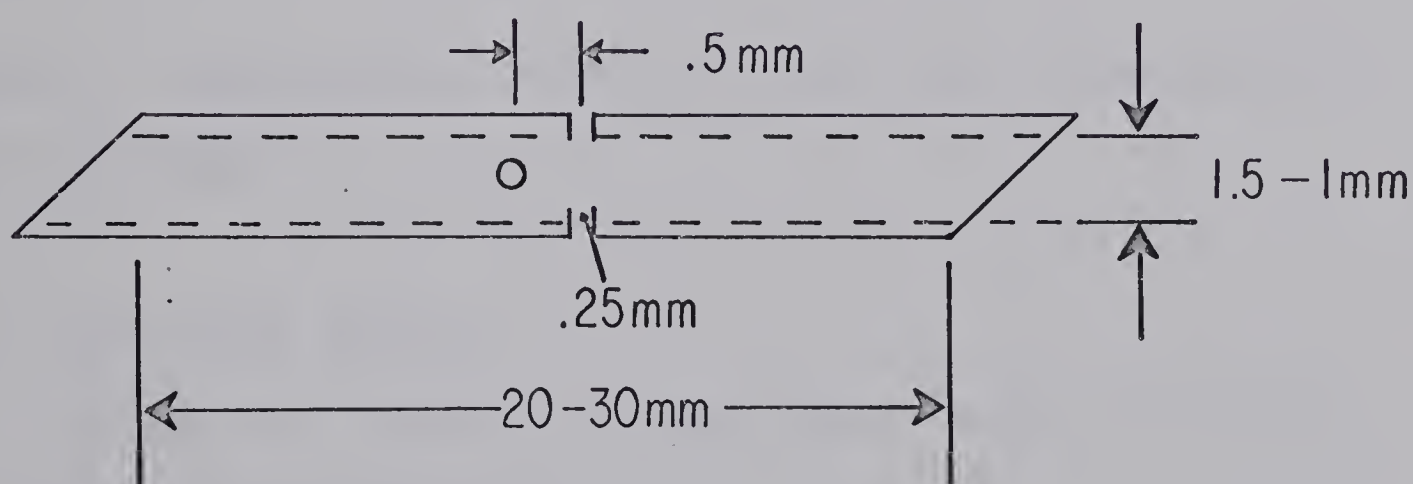


Figure 2. Glass tube used for probe (not to scale).



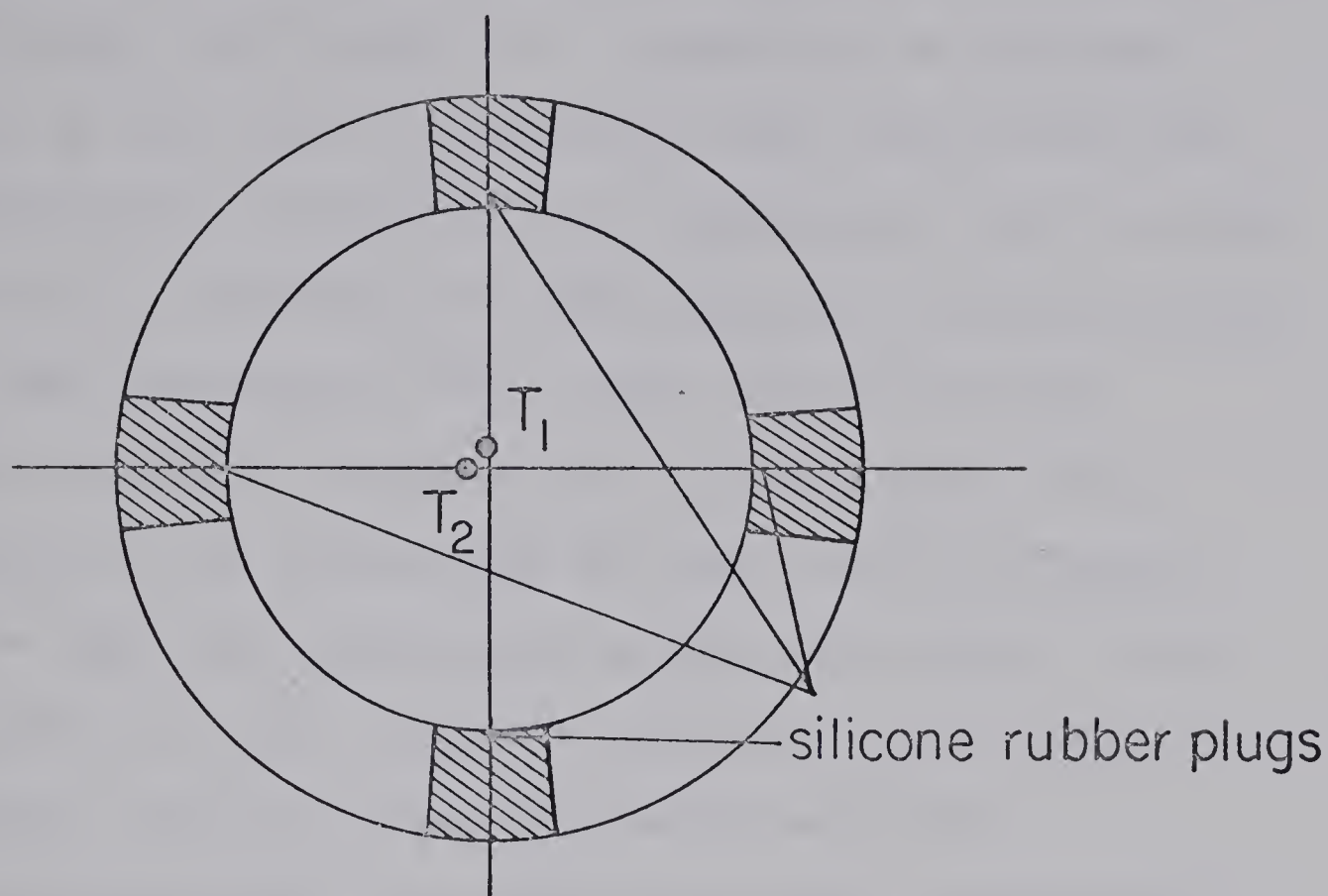


Figure 3. Approximate location of the two thermistors in the glass tube.

## 2.6 Flow Meter Scheme

The general scheme of a flow meter using thermistors falls into two categories:

- (1) constant current
- (2) constant temperature.

For this project the constant temperature technique was chosen in order to decrease the time constant of the thermistor. This technique consists of a scheme in which the power is adjusted automatically using negative feed-



back to keep the temperature, and hence the resistance of the measuring thermistor, constant. This system works well for hot wires, and simple D.C. systems may be designed. However, in the case of thermistors with their high negative temperature coefficients of resistance, a D.C. system results in an inherently unstable positive feedback system. Grahn, Paul and Wessel (1968, 1969) claim they have achieved stability and speed with a D.C. system. An evaluation of the system used by Grahn, Paul and Wessel indicates that the system gain is less than unity. This would result in their achieved stability but it leaves in doubt their claim for improved system bandwidth.

In this project a technique originally suggested by Ziegler (1934) was used. He suggested that the heating current and the error voltage be separated. In a system which measures the resistance of the thermal sensor by means of D.C. and which heats the sensor by means of RF power, one can obtain such a system in which heating does not influence the measurement. Such a system has never been designed. The scheme suggested by Ziegler to improve the stability of hot wire systems, is essential for a thermistor system because of the negative temperature coefficient of resistance of thermistors.

The system used in this project consisted of a high frequency oscillator which was amplitude modulated. The amplitude modulator drives a power amplifier which in turn drives the thermistor. A D.C. bias current through







the thermistor is used to indicate changes in resistance. This change in resistance causes the error voltage which is amplified and in turn changes the amount of RF power fed into the thermistor. The output voltage of the amplifier is then a function of velocity. Figure 4 is a basic scheme of the proposed forced convection flow meter.

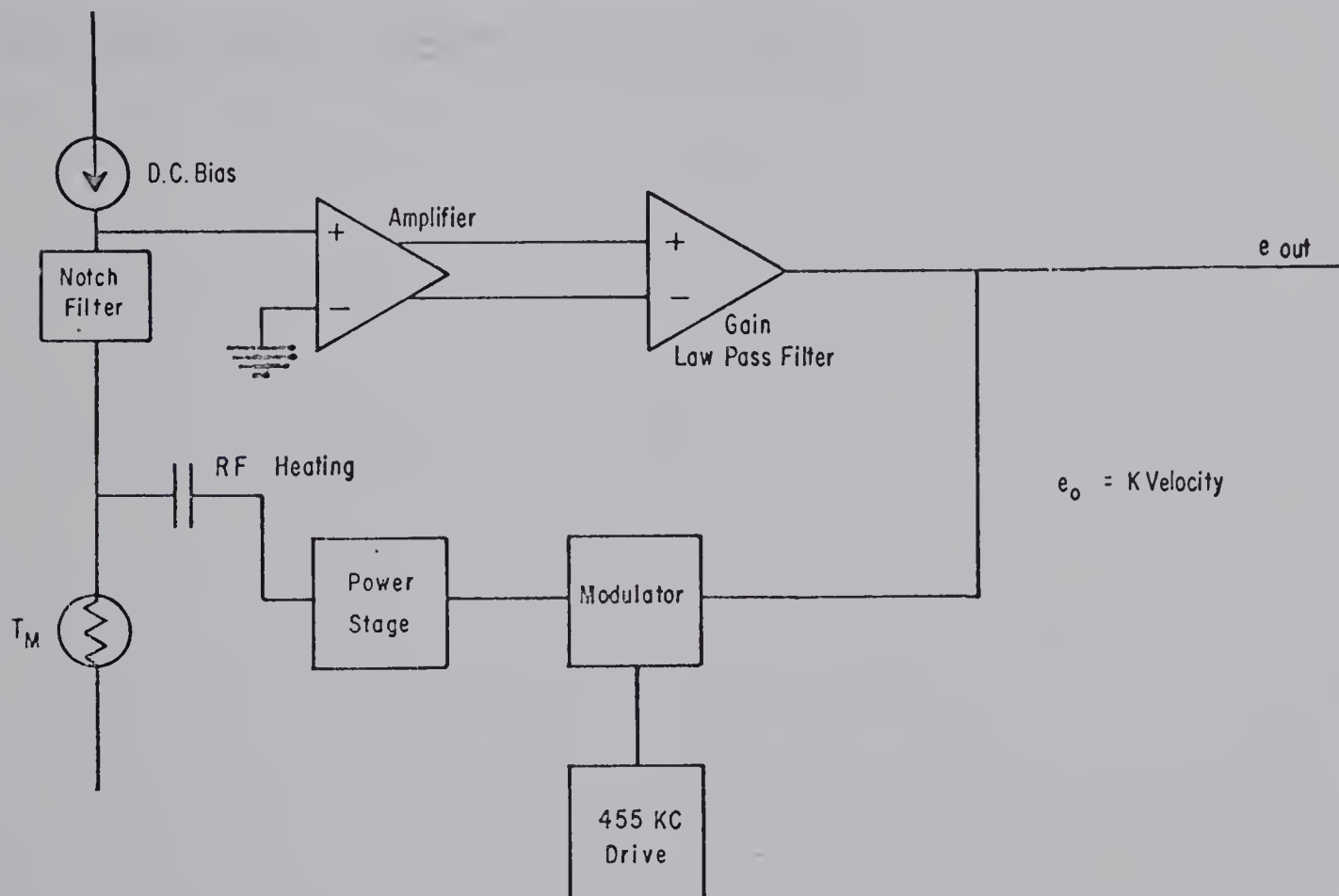


Figure 4. Basic scheme of flow meter.



## 2.7 Thermistor Biasing

The measuring thermistor must be heated above the ambient temperature of the fluid so that it is sensitive to velocity. This requires some bias power. The bias power must be large enough so that the thermistor is in the self-heated portion of its characteristic but not so large that it is in the negative resistance portion of its characteristic (Figure 1). The temperature of the thermistor in this case must also be below the temperature that would cause damage to the blood.



## Chapter 3      STEADY STATE RESPONSE OF FLOW METER

### 3.1 Introduction

If a heated body such as a thermistor is placed in a fluid stream and kept at a constant temperature above the ambient temperature of the fluid, power is required to maintain it at that temperature. This power depends on the fluid velocity, the specific heat of the fluid, the temperature difference and the thermal conductivity of the fluid. Therefore, once the thermistor is biased at some temperature above the ambient temperature of the fluid, the additional power required to keep the thermistor at a constant temperature when forced convection is present (i.e. non-zero velocity) is a function of the velocity of the fluid at the point of measurement.

### 3.2 Calibration of Probe

The measuring thermistor was biased at  $4^{\circ}\text{C}$ . above the ambient temperature of the fluid, which was  $37^{\circ}\text{C}$ ., using the scheme shown in Figure 5. (Detailed schematic diagrams of the individual blocks are shown in Appendix 2.) The resistance of the thermistor at this level of heating (8 mw) was about 1.2 Kohm.

The unheated reference thermistor  $T_R$ , shown in Figure 5, did not provide adequate temperature compensation at this stage, so the syringe driver and the probe were immersed in a constant temperature bath which was maintained at  $37^{\circ}\text{C}$ .



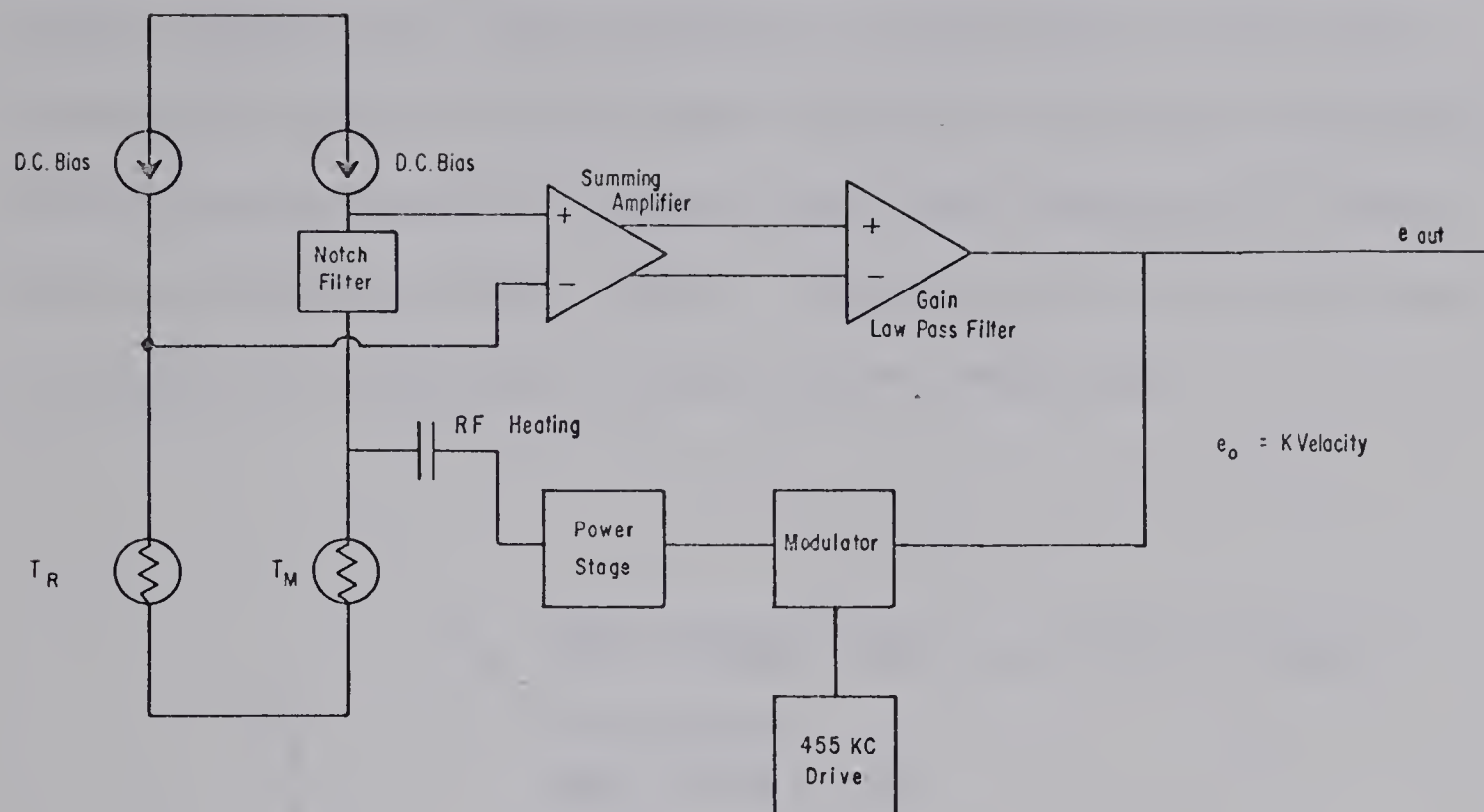


Figure 5. Initial flow meter scheme used for calibrations.

Using 0.16M saline as the fluid and a syringe driver to provide variable flow rates, the calibration curve shown in Figure 6 was obtained. Average velocities were calculated from the flow rates.

The slope of the curve shown in Figure 6 is  $\frac{1}{4}$  (0.26). From this curve it is evident that the output voltage of the amplifier is directly proportional to the fourth root of velocity. This rapidly saturating non-linearity can, of course, be broken down into two square root functions in series. From the scheme shown in Figure 5 it is obvious that one square root non-linearity is due to the second power of current in Joule's Law. This is due to the fact





that the power fed into the thermistor is proportional to the square root of the output voltage of the system. The other square root non-linearity is then due to the non-linear forced convection heat transfer from the thermistor. This function does not agree with that obtained by Grahn, Paul and Wessel (1968, 1969). They reported that the heat transfer is a function of the log of velocity.

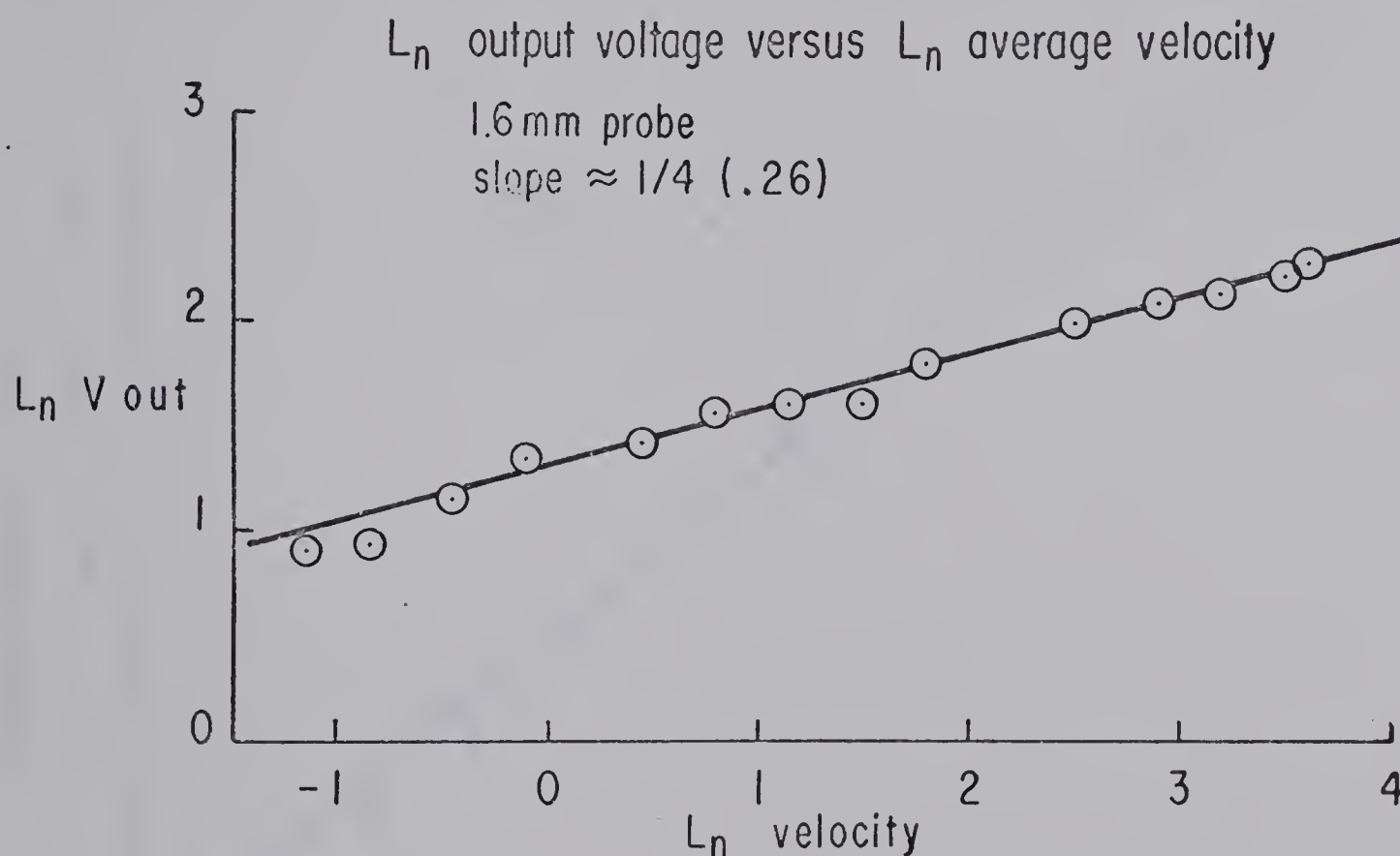


Figure 6. Calibration curve for 1.6 mm. diameter probe. The natural log of output voltage is plotted against the natural log of average velocity.

The system shown in Figure 5 is a regulator (input is zero). Therefore the square root function, due to Joule's



Law, in the forward path may be cancelled by insertion of a similar non-linearity in the feedback path. This modification is shown in Figure 8. A square root function was generated using a diode network and an operational amplifier. Another operational amplifier was used to eliminate sign inversion and provide the offset for the bias power to the measuring thermistor. The gain of the second amplifier was set at  $\frac{1}{2}$  to allow the non-linearity to operate over a 10 volt range.

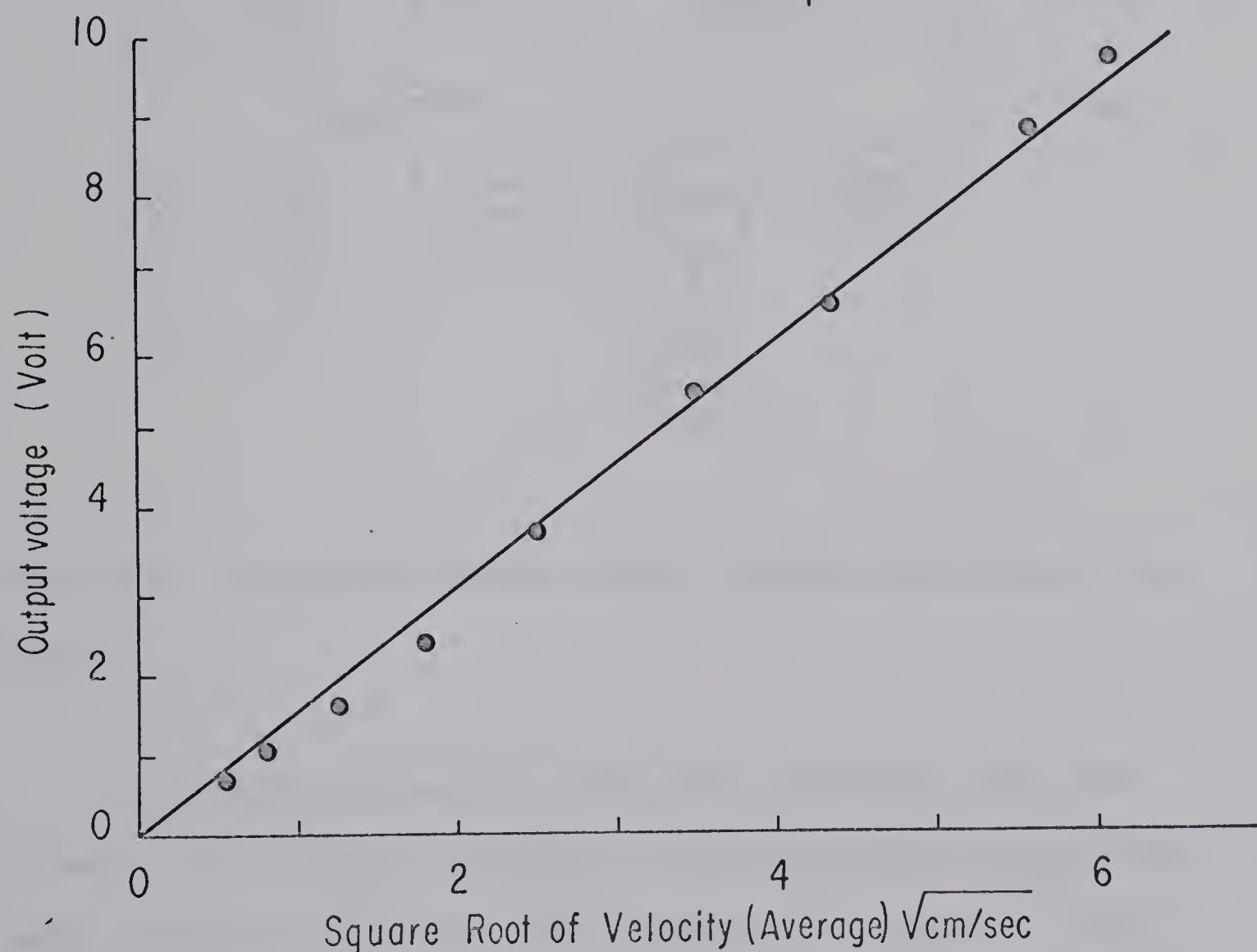


Figure 7. Calibration curve of 1.6 mm. diameter probe.

The flow meter scheme is the same as shown in Figure 5 except that a square root non-linearity has been inserted in the feedback path. The output voltage is plotted versus the square root of velocity.



A detailed schematic of the square root scheme is shown in Figures A-10 and A-11.

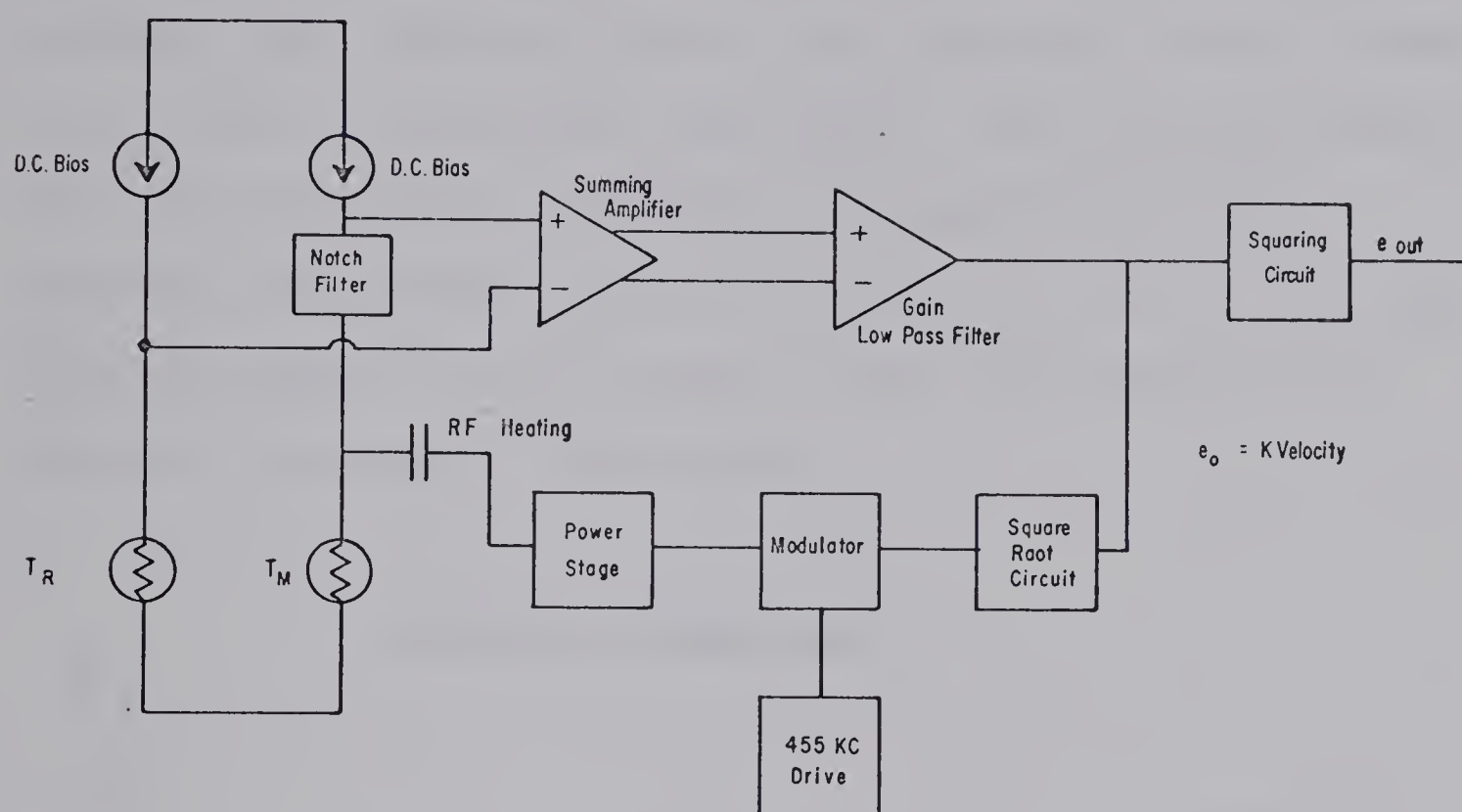


Figure 8. Complete scheme of the forced convection flow meter.

The additional modification was inserted into the scheme, as is shown in Figure 8, and a second calibration was performed using the same procedure as before. This was done in order to check the calibration deduced previously. A calibration curve is shown in Figure 7. The output voltage is now directly proportional to power.

The amount of heat transferred from the thermistor is



a function of the square root of velocity as is shown in Figure 8. Since it was essential that the output voltage of the flow meter be proportional to velocity, further linearization was required. This linearization was easily obtained by inserting a function which squares the output voltage. The squaring function was generated using a diode network and an operational amplifier. This function generator was preceded by a variable gain amplifier which amplified the output signal as required so that at low flow rates the entire 10 volt range of the non-linearity was utilized (Figures A-12 and A-13).

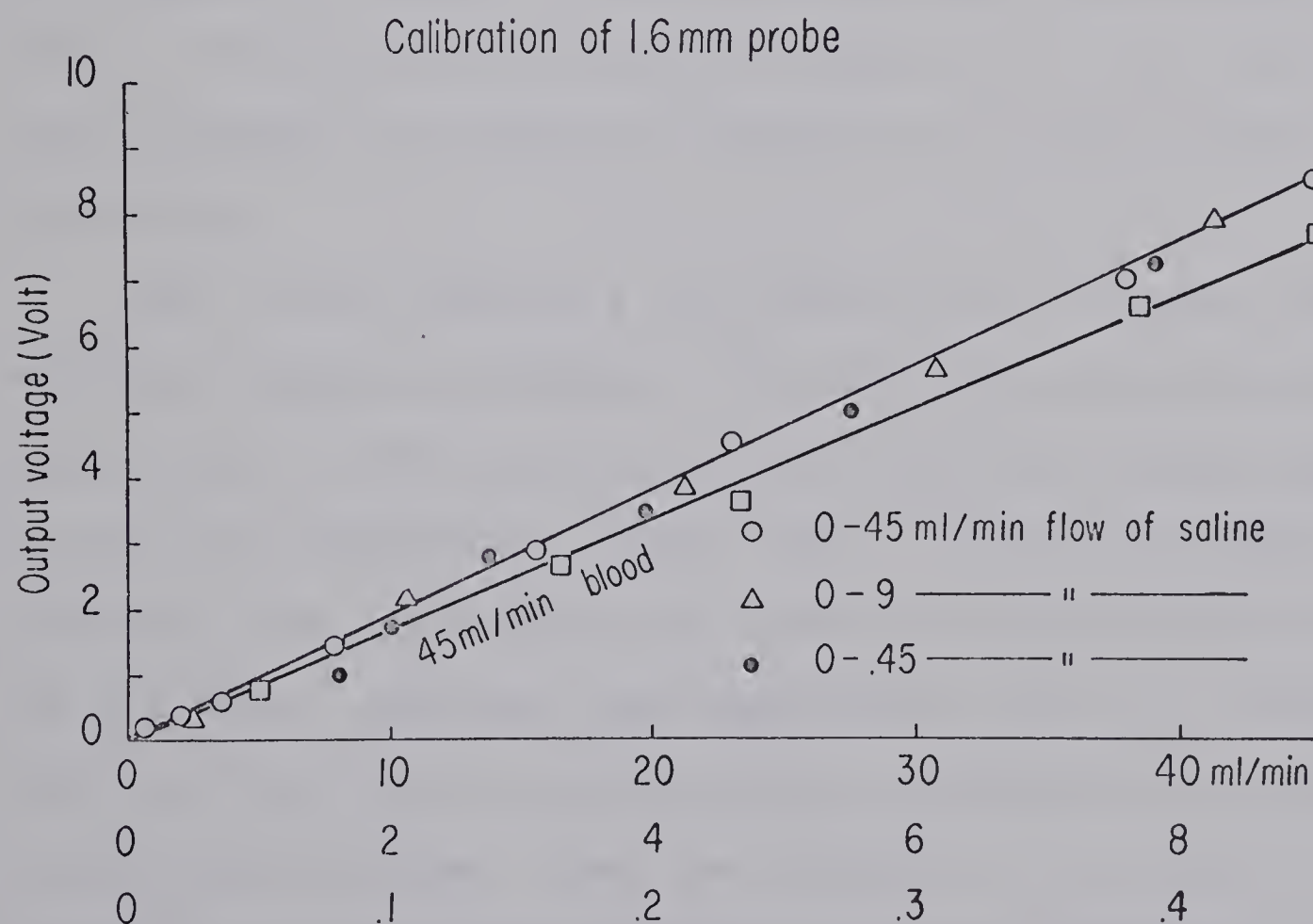


Figure 9. Calibration curves for the forced convection flow meter. Whole dog blood or 0.16M saline was used as fluid.





The complete scheme of the forced convection flow meter is shown in Figure 8. Calibration curves were obtained for the flow meter using 0.16M saline and whole dog blood. These curves are shown in Figure 9.

### 3.3 Theoretical Calculation of Heat Transfer

It has been stated before that the output of the controlling amplifier which is a measure of the power required to keep the temperature of the thermistor constant is also a function of velocity. In steady uniform isothermal flows the rate of convection heat loss from a solid body depends not only on the flow velocity and body geometry, but also on the physical properties of the fluid and the difference in temperature between the fluid and the solid body.

King (1914) derived a law relating heat transfer and velocity in small cylinders. This law is an extension of Boussinesq's (1905) earlier work and has been widely applied to hot wire anemometry. King's Law is derived assuming potential flow ( $\text{viscosity} = 0$ ) which is a good approximation for air flow. However, when considering flow of a viscous fluid such as blood or 0.16M saline, potential flow is not a good approximation. When the viscosity of a fluid is non-zero, a fluid layer adjacent to a wall adheres to it. The relative velocity between the layer of fluid and the wall is zero. This type of flow is called boundary layer flow where the boundary layer thickness is defined as the



distance from the wall to where the velocity approaches 99% of the free stream velocity.

Before an attempt was made to calculate the heat loss from the thermistor, some simplifying assumptions were made. They are as follows:

- (1) The heat loss is calculated on the basis that the thermistor is spherical in shape.
- (2) The heat loss from the platinum wires is small and thus neglected.
- (3) The RF power goes into the thermistor and none is dissipated by the blood which is an ionic fluid (possible error eliminated by measurement).
- (4) The effect of radiation to the fluid is small and may be neglected since temperature differences are small.
- (5) The coefficient of heat conduction of the thermistor of radius  $r_0$  is very large compared with that of the surrounding medium so that the temperature of the sphere may be taken as uniform.
- (6) The thermistor does not distort the flow field.

The equation of heat transfer (Landau and Lefshitz, 1959) in an incompressible fluid is as follows:

$$\frac{\partial T}{\partial t} + \mathbf{V} \cdot \text{grad } T = \frac{\kappa}{\rho C_p} \nabla^2 T \quad \dots (3.1)$$



where  $T$  = the temperature field

$t$  = the time

$V$  = the free stream velocity of the fluid

$\kappa$  = the thermal conductivity of the fluid

$C_p$  = the specific heat of the fluid and

$\rho$  = the density of the fluid.

This equation neglects the temperature variation of  $\kappa$ ,  $C_p$  and  $\rho$  which is valid since the temperature difference between the thermistor and the fluid is small. In calculating the heat transfer as a function of velocity, only steady convective flow is important and all time derivatives can be set to zero. It follows that

$$V \text{ grad } T = \frac{\kappa}{\rho C_p} \nabla^2 T. \quad \dots (3.2)$$

In spherical co-ordinates, equation (3.2) becomes:

$$V_r \frac{\partial T}{\partial r} + \frac{V_\theta}{r} \frac{\partial T}{\partial \theta} = \quad \dots (3.3)$$

$$\frac{\kappa}{\rho C_p} \left( \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 \frac{\partial T}{\partial r}) + \frac{1}{r^2 \sin \theta} \frac{\partial}{\partial \theta} (\sin \theta \frac{\partial T}{\partial \theta}) \right).$$

For flow around a sphere a two dimensional description of both temperature and velocity fields is sufficient since

$$\frac{\partial T}{\partial \phi} = \frac{\partial^2 T}{\partial \phi^2} = 0.$$

The  $\theta$  and  $r$  components of velocity at low velocities (boundary layer flow) are

$$V_r = V \left( 1 - \frac{3}{2} \frac{r_0}{r} + \frac{1}{2} \left( \frac{r_0}{r} \right)^3 \right) \cos \theta \quad \dots (3.4)$$



$$V_{\theta} = -V \left( 1 - \frac{3}{4} \frac{r_0}{r} - \frac{1}{4} \left( \frac{r_0}{r} \right)^3 \right) \sin \theta \quad \dots(3.5)$$

where  $r_0$  = the radius of the sphere and

$V$  = the free stream velocity.

Substitution of (3.4) and (3.5) into (3.3) results in a partial differential equation that is very difficult to solve. Numerical or successive approximation techniques are the only methods which will yield any type of solution.

Kudryashev and Smirnov (1965), using a simplified version of equation (3.3), obtained an approximate solution in closed form. Their solution for the heat transfer from a sphere (held at a constant temperature) due to forced convection is

$$Nu = 0.43 \sqrt{Re} \sqrt{Pr} \quad \dots(3.6)$$

where  $Nu$  = the Nusselt number

$Re$  = the Reynolds number and

$Pr$  = the Prandtl number.

$$Nu = \frac{P 2r_0}{\kappa \Delta T} \quad \dots(3.7)$$

$$Re = \frac{V 2r_0}{\nu} \quad \dots(3.8)$$

$$Pr = \frac{\nu \rho C_p}{\kappa} \quad \dots(3.9)$$

where  $P$  = the power dissipation per unit area in milliwatts per  $\text{cm}^2$  and

$\nu$  = the kinematic viscosity.

When the temperature difference and body geometry of the thermistor is taken into account, equation (3.6) may be







written as:

$$P_T = 0.74 \sqrt{V} \quad \dots (3.10)$$

where  $P_T$  = the total heat transferred in milliwatts.

The approximate solution obtained by Kudryashev and Smirnov (1965) is plotted in Figure 10. The plot in Figure 10 is for a temperature difference of  $4^\circ \text{C}$ . Also shown in Figure 10 is the calibration curve (best fit straight line) from Figure 7 plotted as a comparison. Considering the assumptions which are involved in deriving the differential equation and solving it, the two curves agree reasonably well.

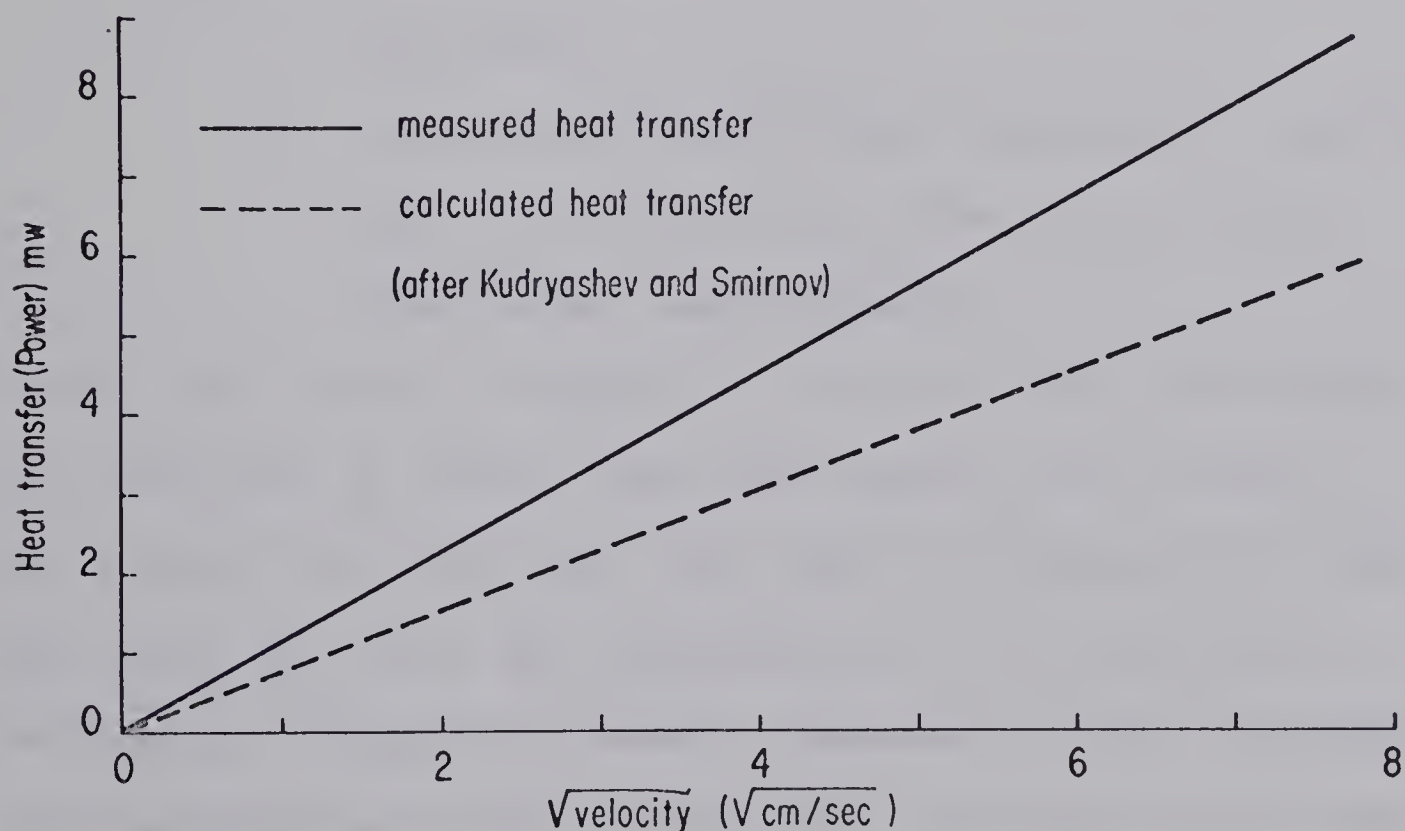


Figure 10. Comparison of theoretically calculated heat transfer from a constant temperature thermistor to the measured heat transfer.



The average velocity used in Figure 7 was corrected for a parabolic velocity profile before being plotted in Figure 10. The average velocity of a fluid calculated from the flow rate in a tube is  $\frac{1}{2}$  that of the maximum velocity at the center of the tube. The velocity at the thermistor was about 90% of the maximum velocity since the measuring thermistor was staggered off center as is shown in Figure 3.

The difference in the two curves shown in Figure 10 is probably due to several factors. They are:

- (1) error in the temperature difference
- (2) error in the dimension of the thermistor
- (3) dissipation of a portion of the power in the fluid
- (4) simplified form of the differential equation used in obtaining the theoretical curve (Kudryashev and Smirnov).

However the curves in Figure 10 indicate that the calibrations obtained by Grahn, Paul and Wessel are in error. This follows from the fact that their system gain is less than unity and hence the thermistor is not operating in the constant temperature mode. Rasmussen (1962) obtained a calibration curve for a thermistor operated in the constant current mode. His results agree with the results obtained by Grahn, Paul and Wessel. Both sets of results exhibit a logarithmic relationship between heat transfer and velocity. Figure 11 shows the measured heat transfer



due to forced convection obtained in this work. This is compared with the heat transfer due to forced convection reported by Grahn, Paul and Wessel (1968).

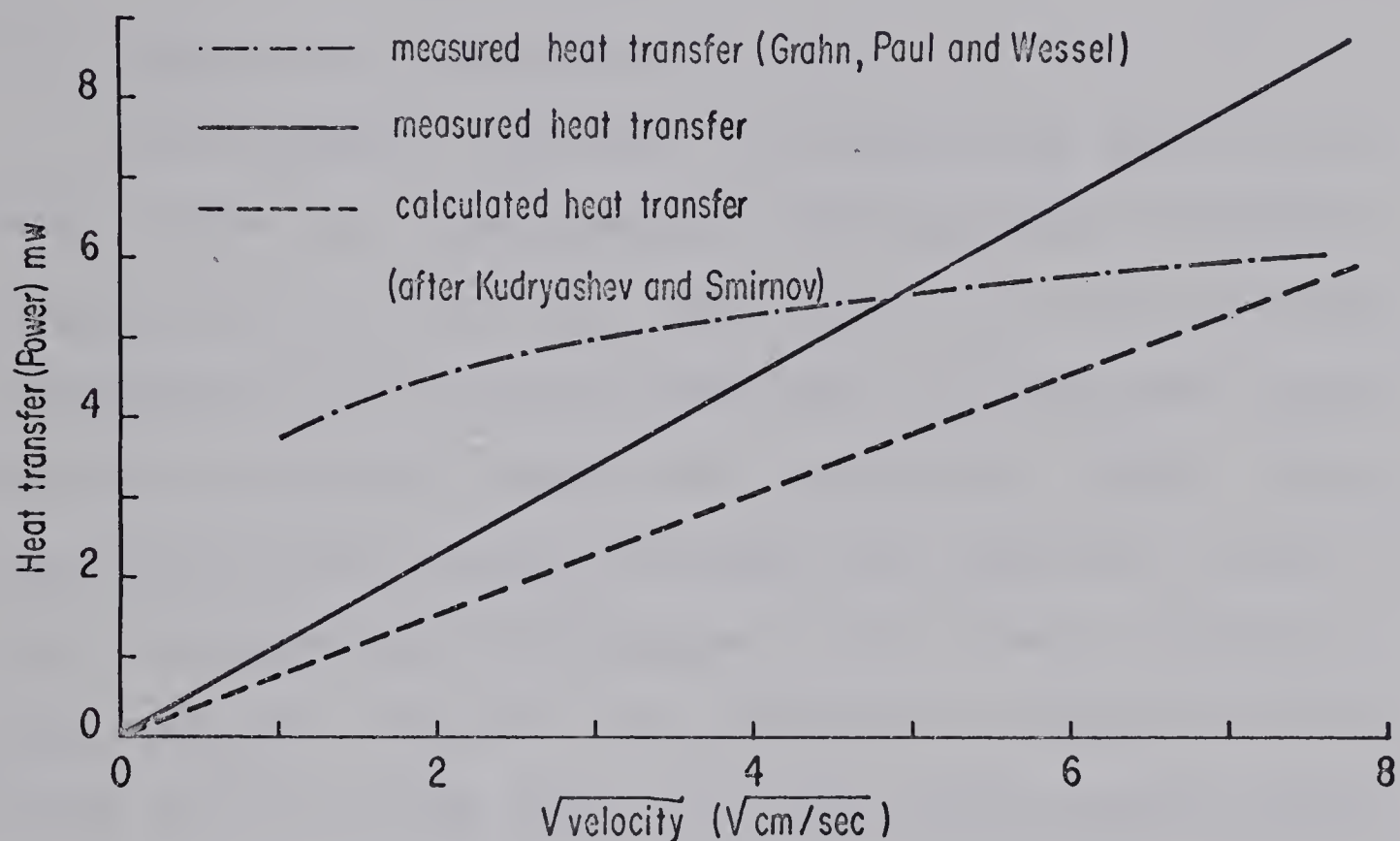


Figure 11. A comparison of the calibration curve obtained in this work to the calibration curve reported (adjusted for similar conditions) by Grahn, Paul and Wessel (1968). Heat transfer is plotted versus the square root of velocity. Also shown is the theoretical curve calculated from the equation (3.10).

The curve obtained by Grahn, Paul and Wessel was corrected before it was plotted in Figure 11. Adjustment of their results was required because the temperature difference used in their calibration was  $13^{\circ}\text{C}$ . and the





dissipation constants of the thermistor used in their work was 40% less than that of the thermistor used in this work. Also shown in Figure 11 is the calculated result using the equation derived by Kudryashev and Smirnov (1965).

### 3.4 Temperature Compensation

The reference thermistor is biased with about  $\frac{1}{2}$  the value of the D.C. bias used for the measuring thermistor. It receives no RF heating. Because of the very low power dissipation the reference thermistor is relatively insensitive to velocity (James, Paul and Wessel, 1966). This insensitivity to velocity follows from equation (3.10). When the temperature difference is zero there can be no heat transfer. The D.C. bias through the reference thermistor can be varied using a 10 turn potentiometer which varies the supply voltage to the current source. The current source is a resistance of 82 Kohm. The variation in current to the reference thermistor is used to provide a balance for zero velocity in the probe. Thermistor characteristics vary with time (aging) and thus this balance must be checked periodically.

Since the measuring thermistor is heated with 8 mw of power and the reference thermistor is unheated, the temperature coefficients of resistance differ in the two thermistors. Therefore, as the temperature of the fluid changes, the reference thermistor will not provide a proper reference signal.





A parallel resistor across the measuring thermistor was used to compensate for differences in temperature sensitivity  $\alpha$ , where  $\alpha$  is derived from equation (2.1) and

$$\alpha = \frac{1}{R} \left( \frac{dR}{dT} \right) = -\beta \frac{1}{T^2} . \quad \dots (3.11)$$

This parallel resistor was picked by trial and error. Figure 12 shows the error in zero balance of the flow meter due to temperature deviations from 37° C. The temperature tracking of the two thermistors is good over a temperature range of 4°. A 10 mV offset would be equal to an error which is 10% of full scale on the most sensitive flow range (0.45 cc./min.). Periodic checks of the temperature compensation scheme indicate that  $\alpha$  is relatively insensitive to aging.

Figure 12 shows that the compensation is valid for a small temperature range. The error becomes greater as the temperature is greatly changed from the balance condition at 37° C.

Another possible source of error in velocity measurements was due to the actual change in resistance of the measuring thermistor due to temperature. This changes the temperature difference between heated thermistor and fluid. For example, if the temperature increases, the resistance of the thermistor decreases, but the power dissipated increases. However, over a small temperature range of up to 2 degrees, the error would be in the order of 2% of full scale flow (0.45 cc./mm.).



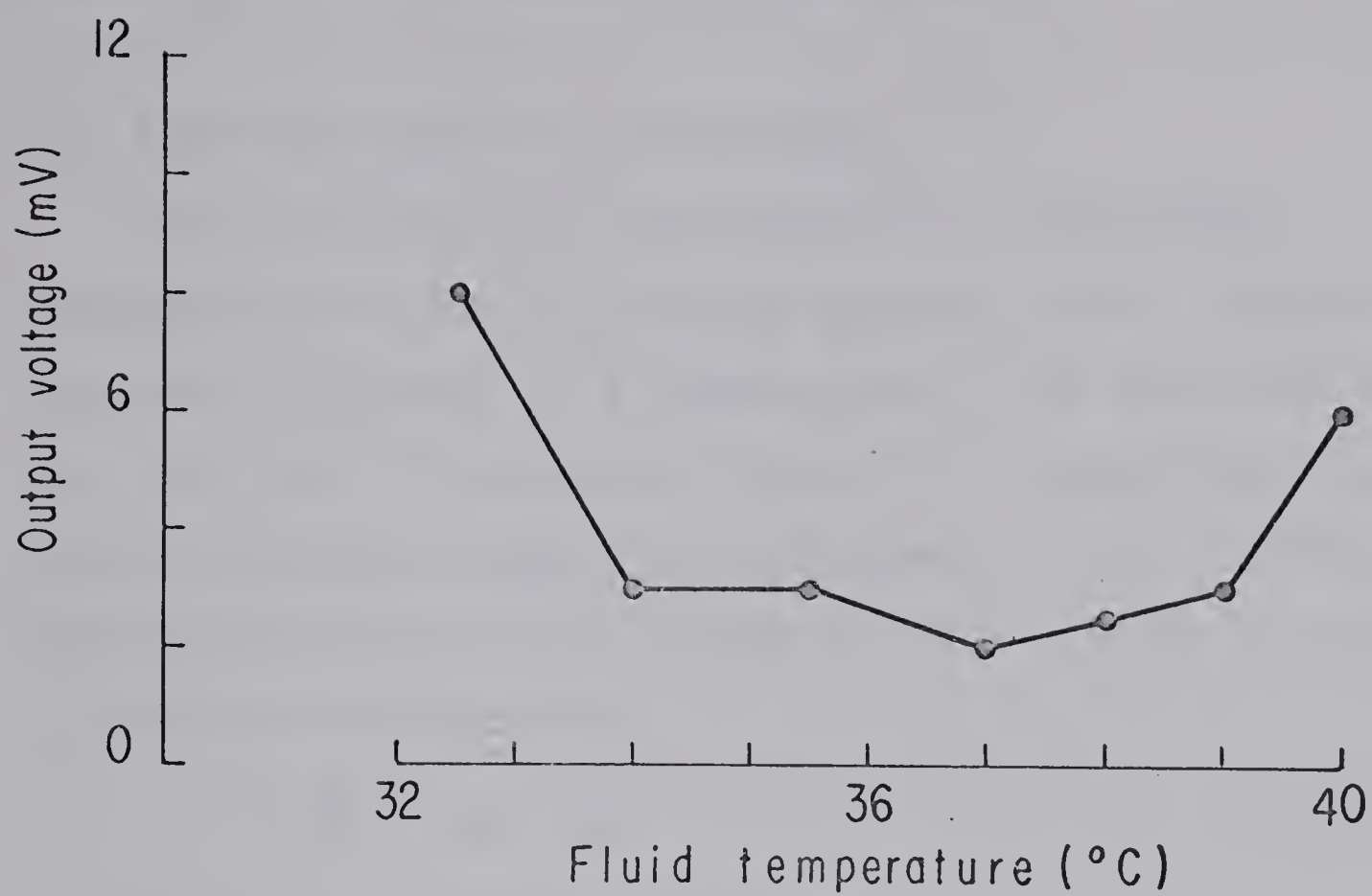


Figure 12. Error in zero balance versus temperature deviations from 37° C. Zero offset is plotted as output voltage.



#### 4.1 Time Constant of the Thermistor

The resistance of a thermistor as a function of absolute temperature is given by equation (2.1). When a thermistor is heated to a temperature  $T$ , and permitted to cool, the rate of cooling is found to be proportional to the instantaneous temperature difference  $T - T_x$ ,  $T_x$  being the temperature of the surrounding fluid. The heat transfer equation is therefore:

$$C \frac{dT}{dt} = K(T - T_x) \quad \dots(4.1)$$

where  $C$  = the heat capacity and

$K$  = the dissipation factor.

The time constant is defined as:

$$\tau_1 = \frac{C}{K} \quad \dots(4.2)$$

Equations (4.1) and (4.2) apply to either an indirectly heated thermistor or to one in which the dissipation of power is small so as to produce negligible heating. A more general heat transfer equation is:

$$C \frac{dT}{dt} = P - K(v) (T - T_x) \quad \dots(4.3)$$

where  $P$  = the power dissipated in the thermistor.

In general,  $K(v)$  depends on the thermodynamical properties of the fluid and the speed of the fluid relative to the thermistor. The time constant of a thermistor is reduced as a consequence of power dissipation in the thermistor and



the velocity of the surrounding fluid.

Rasmussen (1962) has shown that the time constant of a self-heated thermistor in a fluid is given by:

$$\tau_2 = \frac{C}{K(v) - P_0 \alpha} \quad \dots (4.4)$$

where  $P_0$  = the initial power specified at  $t = 0$  and

$$\alpha = \frac{-\beta}{T^2}.$$

This agrees with the equation derived by Janssen, Ensing and Van Erp (1959) for a hot wire.

From equations (4.2) and (3.11) the new time constant for a heated thermistor in a fluid stream is given by the expression:

$$\tau_2 = \frac{\tau_1}{\left(1 + \frac{\beta P_0}{T_0^2 K(v)}\right)} \quad \dots (4.5)$$

where  $\beta$  = a constant which depends on the material used to make the thermistor.

The function  $K(v)$  is a function of velocity. In Chapter 3 it was shown by measurement that:

$$K(v) = 2 + 0.225 \sqrt{V} \text{ mw./degree} \quad \dots (4.6)$$

where 2 mw./degree is the amount of power required to provide the initial bias of temperature difference between the thermistor and the fluid. Figure 13 is a signal flow diagram of the forced convection flow meter without the output squaring circuit.





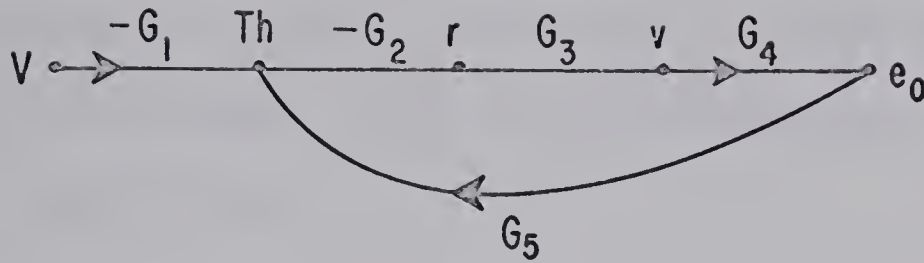


Figure 13. Signal flow diagram of forced convection flow meter.

The transfer functions (using Laplace transformation) in Figure 13 are:

$$G_1 = \frac{0.9}{\sqrt{V}} \quad \dots (4.7)$$

$$G_2 = \frac{K_1}{1 + s\tau_2} \quad \dots (4.8)$$

$$G_3 = K_2 = 0.1 \times 10^{-3} \quad \dots (4.9)$$

$$G_4 = K_3 = \frac{1 \times 10^4}{(1 + s\tau_3)(1 + s\tau_4)} \quad \dots (4.10)$$

$$\tau_2 \gg \tau_3, \tau_4$$

$$G_5 = K_4 \quad \dots (4.11)$$

The static loop gain  $K$ , is equal to  $K_1 \times K_2 \times K_3 \times K_4$  and was measured to be  $2.1 \times 10^3$ . The transfer function, assuming  $\tau_3$  and  $\tau_4 = 0$ , can be written as:

$$\frac{e_0}{V} = \frac{G_1}{G_5} \frac{K}{K + 1} \frac{1}{1 + s\tau_8} \approx \frac{G_1}{G_5} \frac{1}{1 + s\tau_8} \quad \dots (4.12)$$

where  $\tau_8 = \frac{\tau_2}{K + 1}$ .

Equations (4.11) and (4.12) show that the effective time



constant at zero velocity of the thermistor has been decreased by a factor equal to approximately  $1/K$ . The time constants  $\tau_3$  and  $\tau_4$  were set to further filter the RF heating voltage. They were incorporated into the summing amplifier.

#### 4.2 Bandwidth of System

The static loop gain  $K$ , for the system was measured to be  $2.1 \times 10^3$ . The effective time constant of the thermistor in air is about 0.25 msec. The corner frequency measured using an air pulse, was estimated to be 600 Hz which corresponds well to the calculated value. Attempts to further increase the bandwidth by increasing the system gain was made impossible by instability, indicating that at least another time constant exists. The bandwidth in air could be further extended by inserting a lag network in the feedback path. This has the same effect as a lead network in the forward path. In this way the bandwidth could be further increased to 0.9 KHz before instability occurred. Figure 14 shows the response of the flow meter to a step function of air with a rise time of about 0.22 msec. The limiting time constant in this case is the two output filters (corner frequencies 190 Hz and 160 Hz). The bandwidth of the flow meter with saline (or blood) as the fluid is at least as high as that in air since the effective time constant of the thermistor in saline is less than that in air (equation 4.2).



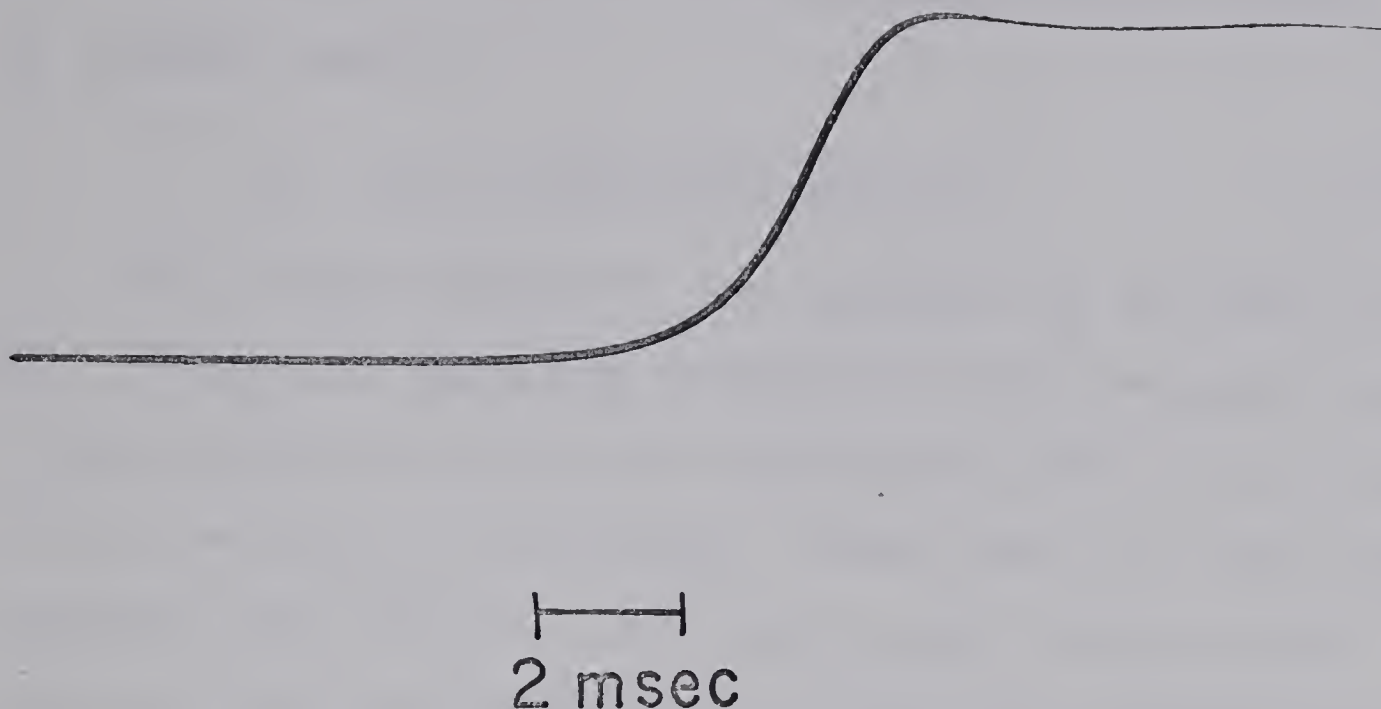


Figure 14. Response of flow meter to step of air flow. (limited by two output filters.) The flow meter was not calibrated for air velocity.

In order to get an approximate idea of the bandwidth of the flow meter with 0.16M saline as the fluid, a small resistor ( $\frac{1}{4}$  ohm) was placed in series with the measuring thermistor. Initially this resistor was shorted. When the short was removed the effective resistance of the measuring thermistor was increased. The system responded with an instantaneous increase in power which came back to balance as the thermistor heated up. The system returned to balance exponentially from which the time constant was estimated. The optimal response of the system was set using the compensating network in the feedback path. The response varied from oscillatory to over-damped.



The transfer function of the compensating network is (Figure A-10):

$$G_5 = \frac{K_4 (s\tau_5 + 1)}{(s\tau_5 + 1)(s\tau_6 + 1) + s\tau_7} \cdot \quad \dots (4.13)$$

The optimal bandwidth was estimated to be about 1.2 KHz. Figure 15 shows the response of the flow meter to a  $\frac{1}{4}$  ohm increase of thermistor resistance with 0.16M saline at zero velocity in the probe. Since this is a much higher bandwidth than required, two first order filters were placed at the output in order to limit the bandwidth at about 125 Hz.

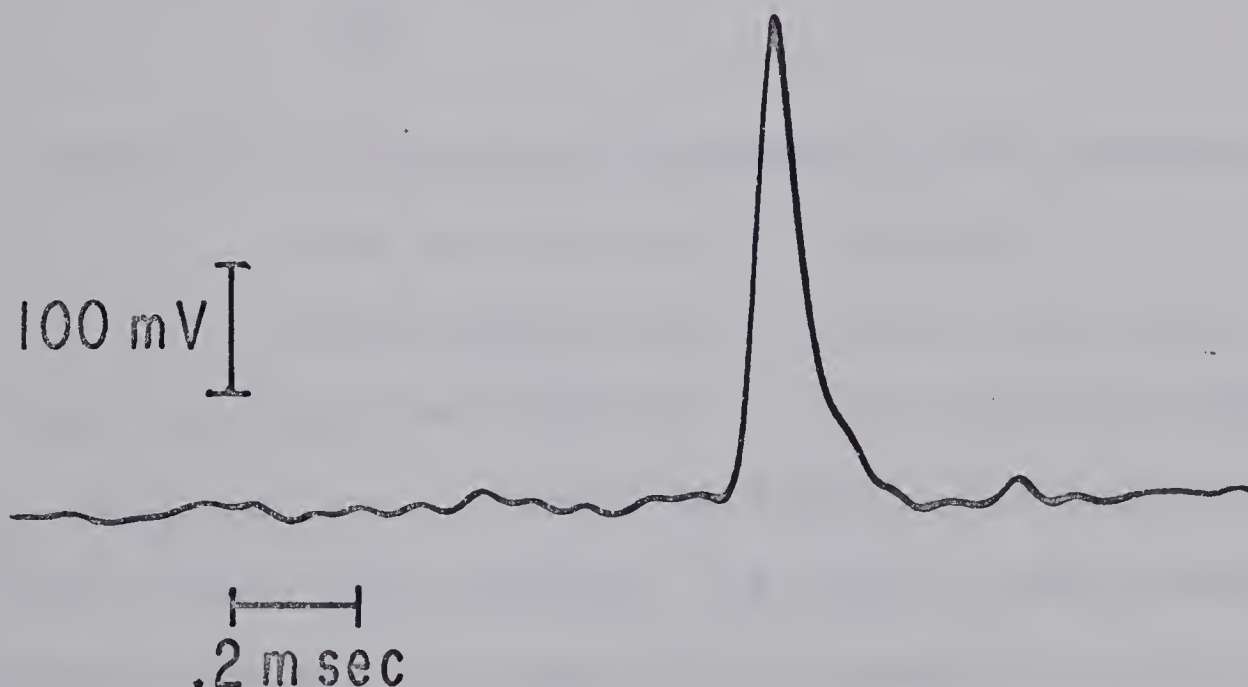


Figure 15. Response of system to a  $\frac{1}{4}$  ohm change of resistance in series with the thermistor.





Figure 15 shows the response actually depends on two time constants. One is the speeded up time constant of the system  $\tau_8$ . The second is slower. It is due to the heat capacity of a layer of fluid next to the thermistor and is also dependent on the velocity of the fluid, since the thickness of the boundary layer decreases with increased velocity.

$$\delta \propto \frac{1}{\sqrt{Re}} \quad \dots (4.14)$$

where  $\delta$  is the boundary layer thickness. This second time constant limits the maximum bandwidth of the system where a fluid such as water or saline is involved. In air it exists but it is much smaller than in saline.

An equation (Corrsin, 1963) which approximates the value of the second time constant is:

$$\tau_9 = \frac{d^2}{D} \quad \dots (4.15)$$

where  $d$  = a function of geometry of the thermistor and the velocity of the fluid and

$D$  = the thermal diffusivity of the fluid.

At zero velocity the value of  $\tau_9 \approx 0.2$  seconds, while  $\tau_2 \approx 0.02$  seconds, so that the limiting bandwidth is set by the slower time constant. If only  $\tau_2$  was present, the maximum bandwidth at zero velocity would be in the order of 15 KHz.

The two time constants are a function of velocity and as a result, the flow meter responds faster to increasing velocities than to decreasing velocities. In part, this is



due to the effect of velocity on frequency response. More important is the fact that the requirement for an increase in thermistor heating with increasing velocity can be met electrically by increasing the heating power. On the other hand, the required cooling of the thermistor cannot be accomplished simply by decreasing the heating power. In addition to a decrease in heating power, cooling requires heat dissipation.

#### 4.3 Stability of the System

The system with a static loop gain of  $2.1 \times 10^3$  was stable. Instability did not occur until the gain was increased to about  $2.5 \times 10^3$ . The system with the square root non-linearity in the feedback path behaves as a linear system. However the heat transfer due to forced convection which is the input is a non-linear function of velocity. The characteristic equation of the system determines its stability. It is:

$$1 + G(s) H(s) = 0 \quad \dots(4.16)$$

where  $G(s)$  = the transfer function of the forward path

and  $H(s)$  = the transfer function of the feedback path.

The system with the compensating network in the feedback path should theoretically be stable for all values of gain. This is true even if the second (postulated) time constant is included. However at very high values of gain, the time constants which were set to filter out the RF power will cause instability. These time constants are  $\tau_3$  and  $\tau_4$ .



Other time constants exist but they are smaller than  $\tau_3$  and  $\tau_4$ .

The root locus diagram is a method of showing the degree of stability achieved. It is a plot in the s-plane of the roots of the characteristic equation of the closed loop system as a function of gain. The underlying principle of this method is based upon the fact that closed loop poles are related to poles and zeros of the open loop transfer function  $G(s) H(s)$  and to the gain. The roots of the denominator of  $G(s) H(s)$  are called poles and roots of the numerator are called zeros when all the coefficients of s are equal to unity.

The characteristic equation of the system, including the second time constant and the compensating network, is given by:

$$1 + \frac{500 (s + 640)}{(s + 5) (s + 50) (s + 1500)} = 0 \quad \dots (4.17)$$

where  $(s = -5)$  and  $(s = -50)$  are the poles due to the thermistor and the fluid. The other pole and zero are due to the compensating network. The compensating network has another pole but it is very large compared to the other poles. A root locus diagram of the system is shown in Figure 16. Also shown in Figure 16 is the effect of another pole at  $s = -80 \times 10^3$  which exists in the system. This one additional pole has the effect of driving the system into instability at higher values of gain. The system contains several such poles and hence this is one reason why the





system becomes unstable. An approximate value gain from the root locus diagram for which the system becomes unstable is  $3.4 \times 10^3$  which is greater than the measured value of  $2.5 \times 10^3$ . This difference in the gains could be due to the effect of other poles at  $s$  very large. Several such poles would cause a decrease in the gain. Contribution of such poles would be small. The major reason for this difference is probably that a one time constant approximation is an over-simplification of the actual lag due to the fluid layer. However the root locus diagram does prove that it is a reasonable assumption.





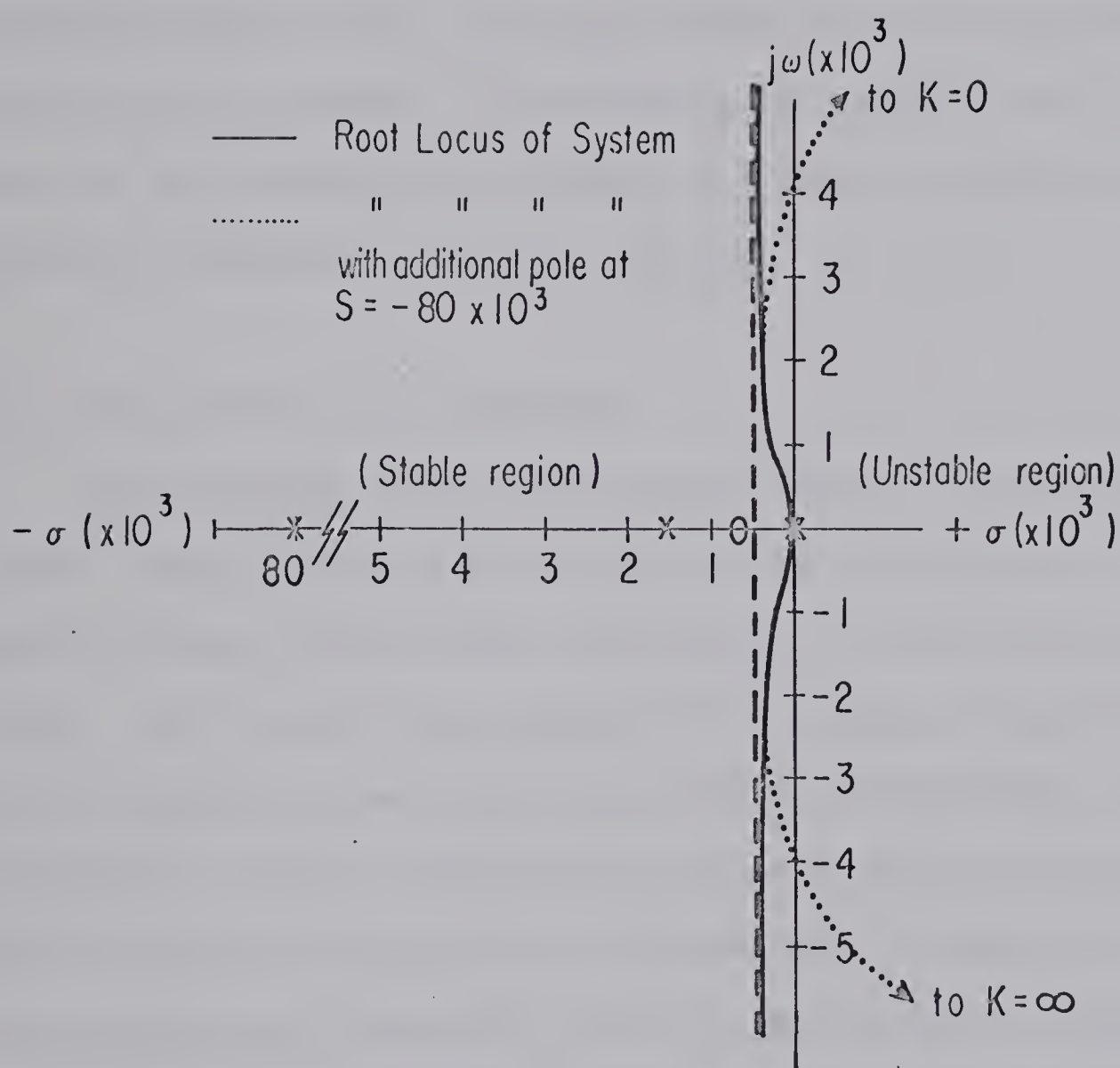


Figure 16. Root loci of the system. The effect on the root locus of a pole at  $s = -8 \times 10^4$  is also shown.



## Chapter 5                    CONTROL OF BLOOD FLOW TO THE                                  SUBMAXILLARY GLAND

### 5.1 Literature Review

Forced convection flow measurement techniques, because of their sensitivity, are applicable to the measurement of blood flow to glands. The control of blood flow to glands, such as the submaxillary gland, has been a controversial problem throughout the last century.

#### 5.1A Blood Flow and Secretion

This problem dates back to the work of Claude Bernard (1858), who presented evidence for the existence of vaso-motor nerves (constrictor and dilator) in the submaxillary gland. He showed that stimulation of the parasympathetic nerve supply to the gland produced vasodilatation, whereas sympathetic nerve stimulation produced vasoconstriction. From this it was generally accepted that vasodilator and vasoconstrictor autonomic nerve fibers ended in close proximity to, and acted directly on, the blood vessels. Later it was observed by Carlson (1907) that sympathetic vasoconstriction was followed by vasodilatation.

Previous to the work of Claude Bernard, Ludwig (1851) had shown that stimulation of the chorda tympani nerve or of the cervical sympathetic chain resulted in secretion of saliva from the submaxillary gland of the cat. The observations by Ludwig were pursued by Heidenhain (1872)



who made the observation that atropine completely blocked the secretion produced by stimulation of the chorda tympani nerve while the vasodilatation was unaffected. Thus for many years it was generally accepted that there were separate secretory and vasomotor nerve fibers in both the parasympathetic and sympathetic nerves to the gland.

The first group to challenge the existence of vasodilator nerve fibers was Barcroft and his co-workers (Barcroft and Müller, 1912; Barcroft and Piper, 1912; Barcroft, 1914) who expressed the view that secretory metabolism in the submaxillary gland determined vasodilatation. Barcroft (1914) concluded (from experiments in which oxygen consumption was measured in the gland before and after atropine) that the increased blood flow was secondary to increased metabolic activity, since increased oxygen consumption, like vasodilatation, was unaffected by atropine. More detailed analysis of Barcroft's work by Bayliss (1923) revealed that the increased metabolic rate and increased vasodilatation produced by nerve stimulation after the administration of atropine were by no means closely correlated. Bayliss maintained that the vasodilatation was controlled by parasympathetic dilator nerve fibers.

Those workers who supported the theory that a relationship existed between blood flow and metabolism did not postulate any chemical mediator whereby the increased





metabolism produced vasodilatation. Werle and von Roden (1936) showed that the potent vasodilator substance, kallikrein, was present in the submaxillary gland and that its effects were atropine-resistant. This led immediately to the suggestion by Ungar and Parrot (1936) that kallikrein was the compound which mediated vasodilatation in the salivary gland when the chorda tympani nerve was stimulated.

For about twenty years little attention was paid to the contention of Ungar and Parrot. However, during the period from 1955 onwards Hilton and Lewis (Hilton, 1960, 1962, 1963; Hilton and Lewis, 1955a, b, 1956, 1957, 1958; Lewis, 1960, 1962, 1963) took up the problem and published a series of papers, not only maintaining the conclusion put forward by Ungar and Parrot, but also suggesting its extension to cover functional vasodilatation in organs generally. A significant generalization has developed from the conclusions of Hilton and Lewis: true parasympathetic vasodilator nerve fibers do not exist. Schachter and his co-workers (Beilenson, Schachter and Smaje, 1965, 1968; Bhoola, Morley, Schachter and Smaje, 1965; Morley, Schachter and Smaje, 1966; Schachter and Beilenson, 1967, 1968) have strongly challenged the conclusions of Hilton and Lewis on the basis of their work on the salivary glands of the cat, rabbit, sheep and dog.

The mechanism of parasympathetic induced vasodilatation in salivary glands, as indicated in the history, is in dispute. Basically, the two theories are:





- (1) parasympathetic cholinergic vasodilator nerve fibers exist, but for some reason produce responses that are atropine-resistant,
- (2) parasympathetic vasodilator nerve fibers do not exist and the vasodilatation that is produced upon parasympathetic nerve stimulation is mediated by kallikrein which is released from the secretory cells.

Hilton and Lewis, supporters of the second theory, maintain that during secretory activity an enzyme, kallikrein, passed from the salivary gland secretory cells into the interstitial fluid where it acts on an  $\alpha_2$ -globulin substrate to release a vasoactive peptide (kallidin). They based their conclusions on the following experimental evidence:

- (1) The output of kallikrein in the venous outflow of a perfused cat submaxillary gland increased greatly upon chorda tympani nerve stimulation, even after atropine had been injected close-arterially, although in the latter case the increase was not quite as great.
- (2) When the chorda tympani nerve was stimulated for the first fifteen seconds of a sixty second arterial occlusion, dilatation occurred when the occlusion was removed;



chorda stimulation during venous occlusion also caused a post-occlusion vasodilatation, but only if the lymph drainage had been removed.

These results were interpreted by Hilton and Lewis as indicating that a metabolic vasodilator material which was normally removed by the lymph system, and which was probably a bradykinin-releasing enzyme (kallikrein), was the mediator of the vasodilatation induced by chorda tympani nerve stimulation. Presumably if the mediator of vasodilatation were acetylcholine, it would have been destroyed during the period of arterial occlusion.

The theory put forward by Hilton and Lewis has been most strongly challenged by Schachter and his co-workers. Bhoola, Morley, Schachter and Smaje (1965) challenged the theory put forward by Hilton and Lewis on the following evidence:

- (1) Close-arterial injection of saliva into a gland with natural circulation failed to duplicate the vasodilatation produced by stimulation of the chorda tympani nerve. The changes in blood flow were slower in onset and were never as large as those produced by nerve stimulation or injection of bradykinin or acetylcholine. The changes produced by saliva were more prolonged.



- (2) After desensitization of the blood vessels to a standard dose of bradykinin, it was found that the response to chorda tympani stimulation or acetylcholine injection was reduced to a lesser degree.
- (3) It was possible to produce a maximal atropine-resistant vasodilatation by stimulation of the chorda tympani nerve when the gland had been perfused by horse serum for as long as three hours. (It had been shown previously that cat saliva fails to release kinin from horse serum.) Under these conditions close arterial injections of dialysed saliva failed to cause vasodilatation.

Beilenson, Schachter and Smaje (1968) found that it was possible to deplete the cat submaxillary gland of kallikrein by chronic ligation of the duct, and by sympathetic stimulation. In such cases, the amount of kallikrein remaining was 0.1% of the control. Such glands nevertheless responded to chorda stimulation with a normal atropine-resistant vasodilatation. Also, they reported that sympathetic nerve stimulation produced saliva with a higher concentration of kallikrein than parasympathetic nerve stimulation.

Several other groups of workers have presented evidence





which challenges the theory that kallikrein plays a role in parasympathetic vasodilatation. Degeneration studies by Kuntz and Richins (1946) showed that extirpation of the superior cervical ganglion resulted in the expected decrease in the number of fibers related to blood vessels; however, considerable innervation still remained.

Snell and Garrett (1958), using histochemical and degeneration techniques, showed that fibers from the chorda tympani nerve supplied branches to ducts and arteries. Electron microscope work by Garrett (1966c, d) on the submaxillary gland of the cat showed that the blood vessels receive a dual parasympathetic and sympathetic innervation.

Terroux, Sekelj and Burgen (1959) showed that moderate doses of atropine depressed salivation as well as the accompanying increase in oxygen consumption without affecting the chorda tympani induced vasodilator response in the dog submaxillary gland. This seems to indicate that the metabolic and vasodilator effects of parasympathetic nerve stimulation may be separated.

Strömblad and Dresel (1963) reported that injection of hemicholinium (HC-3) into the duct of the parotid gland of the cat gradually blocked parasympathetic induced vasodilatation. Hemicholinium gradually decreases the release of acetylcholine by diminishing the resynthesis of acetylcholine in nerve fibers. Work by Prasad and MacLeod (1966) suggests that HC-3 may also alter the sensitivity of the smooth muscle receptors to acetylcholine. These results





support the theory that acetylcholine mediates a significant portion of parasympathetic induced vasodilatation in cat salivary glands; however, they do not eliminate the possibility that acetylcholine only mediates an intermediate step.

Skinner and Webster (1968) showed that the vasodilator effects of chorda tympani stimulation were unaffected in cats pre-treated with carboxypeptidase B (a potent inactivator of bradykinin or kallidin), whereas the equivalent vasodilator effects of kallidin injected close-arterially were abolished. They suggest that the major part of chorda vasodilatation is due to the presence of adrenergic neurones in the chorda tympani nerve which cause vasodilatation via  $\beta$ -adrenergic receptors which are present in the blood vessels. They also report that parasympathetic vasodilatation is suppressed by propranolol (a  $\beta$ -receptor-blocking drug) and that propranolol blocks the vasodilatation produced by isoprenaline injected close-arterially.

These results are challenged by Schachter and Beilenson (1968), who have confirmed the observations of Skinner and Webster (1968) with propranolol, but have also reported a decreased sensitivity to acetylcholine with increased doses of propranolol. Reserpine, which depletes noradrenalin, has been used in cats by Davey, Davies, Reinart and Scholfield (1965), and those authors report that parasympathetic stimulation is unchanged while sympathetic effects are abolished. These results were confirmed by Schachter



and Beilenson (1968).

In 1967 Hilton and Torres repeated the work by Beilenson, Schachter and Smaje (1965) and reported that the kallikrein content drops to only three percent of the control value, and that in such glands there was an increased sensitivity to bradykinin and that the sensitivity to close-arterial injections of acetylcholine was the same as that in normal glands. After further depletion of the glandular kallikrein by stimulation of the sympathetic nerve, Hilton and Torres (1967) reported that the parasympathetic vasodilator response was then reduced.

Hilton (1970) maintains that parasympathetic vasodilatation is mediated by kallikrein and that parasympathetic vasodilator nerves do not exist; on the other hand, Schachter and his co-workers suggest that kallikrein plays no role in parasympathetic vasodilatation. Schachter and his co-workers also suggest that parasympathetic vasodilatation is cholinergic, but is in some way resistant to atropine.

Hilton and Lewis (1956) have extended their theory of kallikrein-kinin mediated parasympathetic vasodilatation to cover the vasodilatation which occurs on cessation of sympathetic nerve stimulation. Meanwhile, Bhoola, Schachter and Smaje (1965) have suggested that the after-dilatation is due to activation of  $\beta$ -adrenergic receptors in the arterioles, and that the kallikrein-kinin system probably



plays no role.

### 5.1B Electrophysiology

Recent electrophysiological work by Creed and Wilson (1969) on the response of secretory acinar cells to nerve stimulation in the submaxillary gland of the cat suggest further experiments which may help resolve the controversy. Bayliss and Bradford (1886) discovered that during secretion an electrical potential could be measured between the hilus and the surface of the gland. They found that the electrical change (electrogram) was abolished by atropine. Bradford (1887) showed that the flow of saliva did not cause the electrogram since compression of Wharton's duct did not affect the electrogram. Moreover, the outflow of saliva after removal of the obstruction did not produce the electrogram. He concluded that the electrogram originated from the glandular cells and that it preceded secretion of saliva.

Further electrophysiological work on the cat submaxillary and sublingual glands was reported by Lundberg (1955, 1957a, b, c, 1958). He used intracellular recording techniques to measure electrical changes in secretory cells of the submaxillary gland and reported a resting potential of -15 to -30 mV. Lundberg (1955) reported that stimulation of either the sympathetic or parasympathetic nerve supply to the gland produced a hyperpolarization, i.e. a secretory potential, across the outer membrane of individual acinar





cells. These hyperpolarizations occurred after a latency period of 0.6 to 1.0 sec. for sympathetic, and 0.2 to 0.4 sec. for parasympathetic nerve stimulation. Lundberg (1955) concluded that a relationship existed between secretory potentials and secretion since, in cases where there was a diminished secretion from a gland after a period of anoxia, or after atropine, the secretory potentials decreased in parallel to secretion. Since Lundberg (1955) was able to show an increase in membrane potential with an increase in strength of single stimuli to the chorda tympani nerve (multiple fiber summation), he suggested that five to ten parasympathetic neurons supplied each secretory cell.

The typical response obtained from the secretory cells was named the type 1 response by Lundberg (1955). He also recorded responses from two other types of cells. The type 2 response had a resting potential of 35 mV. The response to parasympathetic nerve stimulation was a hyperpolarization after a latency of 400 msec. while the response to sympathetic nerve stimulation was a depolarization. Lundberg suggested that the type 2 response originated from the demilune cells. The type 3 response which originated from the duct cells responded to both parasympathetic and sympathetic nerve stimulation with a depolarization after a latency of several seconds.

Garrett (1966c, d) described small extra-acinar





bundles of nerve fibers in close relationship to secretory cells with the smallest distance between a nerve bundle and acinus being 1000 Å. Garrett further suggested that neuro-effector sites might occur where the axons within these bundles become partly free of Schwann cell investments.

Creed and Wilson (1969) report that the hyperpolarization response to preganglionic parasympathetic nerve stimulation and to post-ganglionic sympathetic nerve stimulation was dependent on the frequency of stimulation. The mean latency of the hyperpolarization response to parasympathetic nerve stimulation at 1, 8 and 100 pulses per second was 273, 420 and 252 msec. respectively. The hyperpolarization response to sympathetic nerve stimulation did not occur until the frequency was over 5 pulses per second; the latency was over 420 msec.

Ultrastructure studies by Creed and Wilson (1969) revealed bundles of unmyelinated fibers between acini; some of the axons were free of Schwann cell investment and contained agranular and granular vesicles. Creed and Wilson also revealed that single nerve fibers were found in intra-acinar positions between the membrane of adjacent secretory cells and these axons contained agranular vesicles. The two membranes (axon and acinar cell) were about 160 Å apart. On the basis that the secretory cells respond to single stimuli of the parasympathetic nerves and only to



repetitive stimuli of sympathetic nerves, Creed and Wilson suggest that the nerves which are 160 Å away from the acinar cell are parasympathetic. Using Richardson's (1964) conclusion that cholinergic axons contain agranular vesicles and occasional larger vesicles with an electron dense central area, and that adrenergic nerve fibers contain both agranular vesicles and small vesicles with a dense core, the intra-acinar fibers are suggested by Creed and Wilson to be cholinergic while the extra-acinar nerves may be derived from both a cholinergic and an adrenergic supply.

The paper by Creed and Wilson suggests an experiment which could partially clarify the problem of the control of parasympathetic vasodilatation. If blood flow can be shown to increase within the latency of the hyperpolarization of the secretory cells, then the vasodilatation cannot be secondary to the secretion of saliva. The mechanism for the secondary action would be the release of kallikrein into the interstitial fluid and the subsequent release of kallidin with resulting vasodilatation. If the blood flow changes show a longer latency than hyperpolarization, the results would then not be very conclusive; although these latencies may be due to mechanical factors, such as transport delays within the gland and the time required to change the size of blood vessels due to smooth muscle relaxation. Transport delays within the gland could be due to the large increase in capacity of the gland caused by vasodilatation. Another latency could be due to the release and diffusion



of transmitter, although this would probably be short as has been suggested by Creed and Wilson (1969).

If the latency between nerve stimulation and an increase in blood flow (vasodilatation) is less than a second, it is unlikely that parasympathetic vasodilatation is mediated by an enzyme system such as kallikrein and bradykinin. Time course studies such as these would not be conclusive proof of the existence of parasympathetic vasodilator fibers unless accurate information on the enzyme kinetics involved was also available. However, bio-assay studies on the kallikrein-kinin system seem to indicate a slow system. More conclusive proof of the presence of parasympathetic vasodilator fibers could be provided if electrophysiological events in the smooth muscle cells in the walls of blood vessels could be correlated to parasympathetic and sympathetic nerve activity. The latency of the electrical response of the vascular smooth muscle may well give a final answer to the question of whether parasympathetic vasodilator nerve fibers do exist or not in the submaxillary gland.





## 5.2 Objectives of Present Work

The first objective was to use electrophysiological techniques to measure the membrane potential changes of secretory cells as has been reported by Creed and Wilson (1970) and, if possible, to measure the electrophysiological changes occurring in the smooth muscle cells of the submaxillary gland blood vessels (arterioles) in the cat.

The second objective of this work was to measure the blood flow changes occurring in the submaxillary gland of the cat as a result of parasympathetic nerve stimulation. The measurements of the blood flow changes were to be done utilizing the flow meter described in the first part of this thesis. In the measurement of blood flow particular emphasis was placed on the time course of blood flow changes.





### 5.3 Methods

#### 5.3A Animals and Anaesthesia

Cats weighing 2 to 4 kg. were used. Anaesthesia was induced by chloroform, aerated with 100% oxygen in a closed box. Ether on a face mask was used for temporary maintenance of anaesthesia until a venous cannula was inserted. Anaesthesia was maintained throughout the experiment by chloralose (80 mg/kg. I.V.).

#### 5.3B Nerve Stimulation

##### (a) Parasympathetic

The parasympathetic nerve supply to the submaxillary gland runs in the chorda tympani nerve which is a branch of the chorda lingual nerve. The chorda lingual nerve was exposed in the region where it crosses the submaxillary and sublingual ducts, and was cut as near as possible to its point of exit from the skull.

##### (b) Sympathetic

The cervical sympathetic nerve could readily be exposed and cut in the neck where it runs alongside the vagus nerve.

#### 5.3C Salivary Flow

The submaxillary duct was exposed in the floor of the mouth through the neck, lying medially to the sublingual duct. The submaxillary duct was cannulated with a fine glass cannula, about 5 - 10 mm. rostral to the point



at which the duct was crossed by the chorda lingual nerve. Flow of saliva in the cannula, upon sympathetic or parasympathetic nerve stimulation, was used as an indication of undamaged operation of the nerve supply in both flow and electrophysiological experiments.

### 5.3D Blood Flow

The blood flow (venous outflow) through the submaxillary gland was measured by putting the flow meter (forced convection) in the external jugular vein. The probe of the flow meter was inserted into the external jugular by a double cannulation. All veins draining into the external jugular vein were tied except that from the submaxillary gland. The cats were heparinized with sodium heparin (10 mg/kg. I.V.) and the inside of the probe was siliconized with Siliclad (Clay-Adams), (1 part Siliclad to 20 parts of water) by soaking the inside for 10 minutes. The probe was then forced-air dried with a heat gun. Figure 17 shows a schematic diagram of the experimental set-up used in the blood flow experiments.



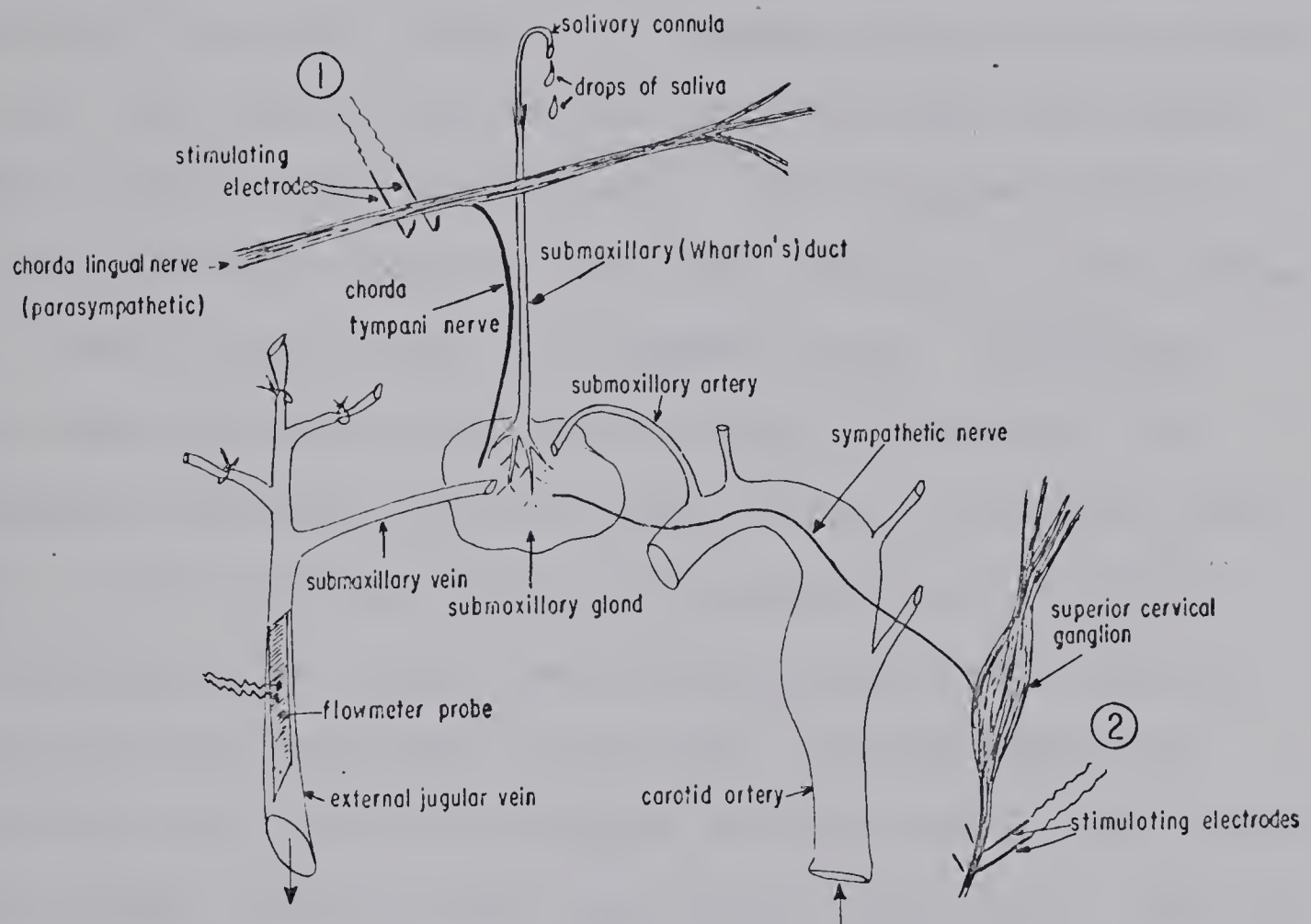


Figure 17. Schematic diagram of the experimental set-up used in the blood flow experiments.



### 5.3E Electrophysiological Experiments

Cats were anaesthetized using chloroform and maintained on chloralose as for blood flow experiments. The sympathetic and parasympathetic nerve supply and the submaxillary duct were prepared as in the blood flow experiments. The submaxillary gland was dissected out carefully to cause little or no damage to the nerve supply. It was taken out of its capsule and the lobes were separated to expose the blood vessels. This exposed portion of the gland was the part which was posterior to the hilus.

The cat was placed in a Faraday cage. Recordings were made with glass micro-electrodes filled with 3 M potassium chloride and were either fixed, or mounted flexibly on fine platinum wire, and connected to a "Zeiss" micromanipulator. When the electrode was fixed, contact was made with chlorided silver wire. The indifferent electrode was a medicine dropper filled with 0.16 M saline and contact was made using chlorided silver wire. Its tip was placed next to the gland. The resistance of micro-electrodes used for the intracellular recording varied from 30 to 55 megohms. The electrodes were connected to a Medistor microelectrode amplifier which had an input impedance of  $5 \times 10^4$  megohms, an input current of less than  $10^{-13}$  amps, and a band width of 10 to 15 KHz with proper neutralization. The output was fed into a Tektronix 565 storage oscilloscope and results were photographed with a polaroid camera. Stimulation was provided with a Grass







SD5 stimulator or with a Digatimer and, unless otherwise indicated, was set at 20 pulses per second and 7 volts with a pulse width of 0.4 msec. Figure 18 shows a schematic diagram of the experimental set-up used for the electrophysiological experiments.

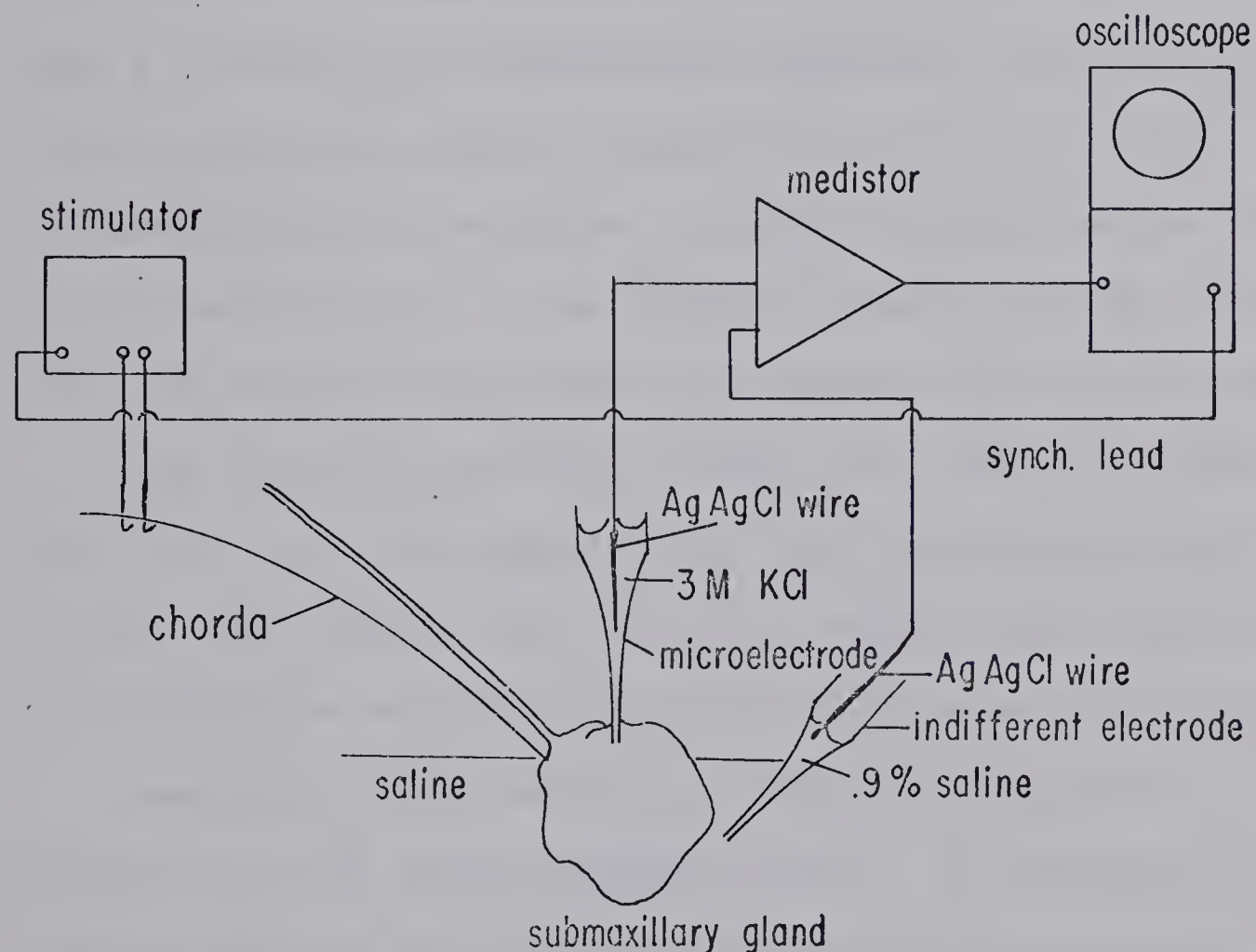


Figure 18. Schematic diagram of experimental set-up used in the electrophysiological experiments.



## 5.4 Results

### 5.4A Electrophysiology

Electrophysiological results obtained from secretory cells in the submaxillary gland agree with the results reported by Creed and Wilson (1969). Figure 19 shows a typical response obtained from a secretory cell with a stimulus of 5 pulses to the chorda tympani nerve (parasympathetic). This type of response was the type 1 response described by Lundberg (1955). He showed that it originated from the outer membrane of secretory cells (acinar). The type 1 response as reported by Lundberg, consisted of a resting potential which varied from 15 mV. to 35 mV. and a hyperpolarization response to chorda tympani nerve stimulation of about 20 mV. The latency between chorda stimulation and hyperpolarization was between 200 and 400 msec.

The resting potential of the cell shown in Figure 19 was 40 mV. and the magnitude of the hyperpolarization was 30 mV. The latency was 500 msec. between the beginning of stimulation and the beginning of hyperpolarization.

Secretory cells were also found to respond to a single stimulus of the chorda tympani nerve. A response to a single stimulus of the chorda tympani nerve is shown in Figure 20. The latency was about 350 msec. and the magnitude of the hyperpolarization was about 12 mV. In this cell the resting potential was 40 mV.



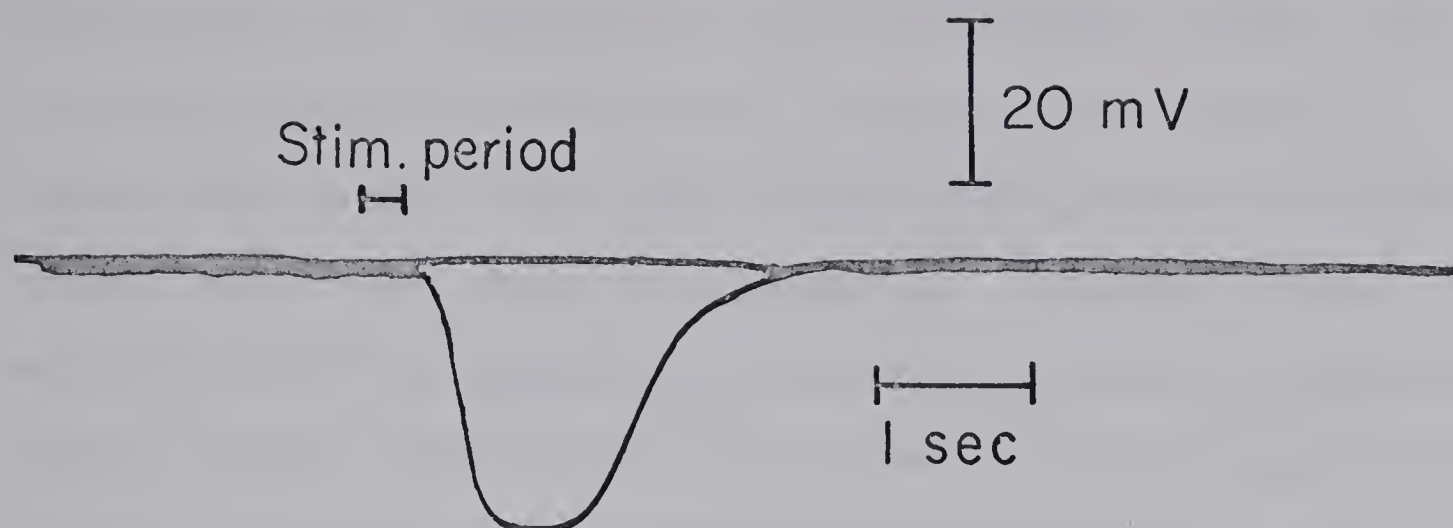


Figure 19. Secretory cell response to 5 stimuli of the chorda tympani nerve.

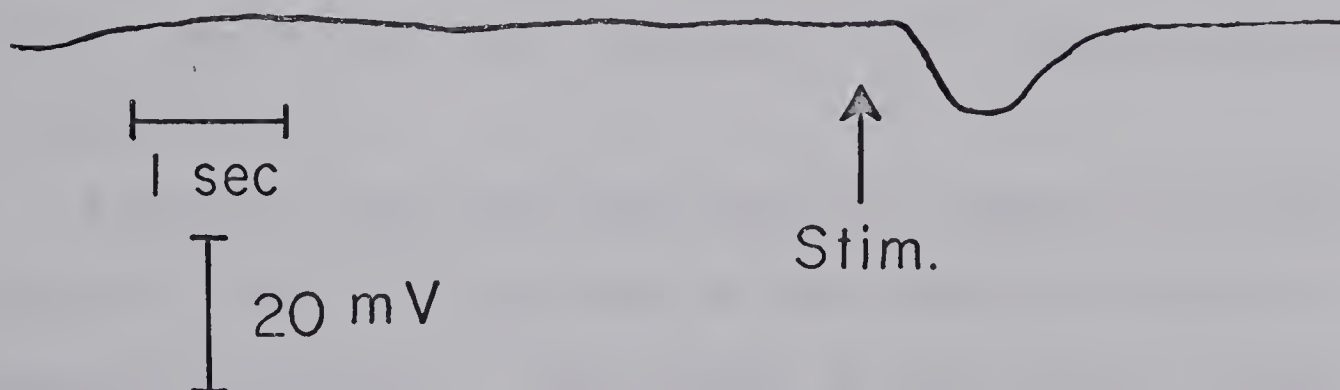


Figure 20. Secretory cell response to a single stimulus of the chorda tympani nerve.



Stimulation of the sympathetic nerve also resulted in a hyperpolarization of secretory cells. This hyperpolarization, as reported by Creed and Wilson (1969), did not occur until the stimulation frequency was nearly 10 stimuli per second and, after an extended period of stimulation, the cells began to depolarize. Lundberg (1955) reported that the sympathetic secretory potential appeared after a longer latency and had a slower rate of hyperpolarization compared to the chorda hyperpolarization. The latency between sympathetic nerve stimulation and hyperpolarization in the submaxillary gland was larger than that observed with chorda tympani nerve stimulation and was nearly a second in some cases. Creed and Wilson reported a mean latency of 662 msec.  $\pm$  178 SD at 10 stimuli per second.

A response of a secretory cell to sympathetic nerve stimulation is shown in Figure 21. The latency is approximately 1 second and the magnitude of the hyperpolarization is about 12 mV.

Figure 22 shows the same type of response as shown in Figure 21, but the magnitude of the hyperpolarization response is greater. The latency in this case is about 800 msec. At the end of the trace the microelectrode was taken out of the cell to give an indication of the magnitude of the resting potential and the direction of potential change.





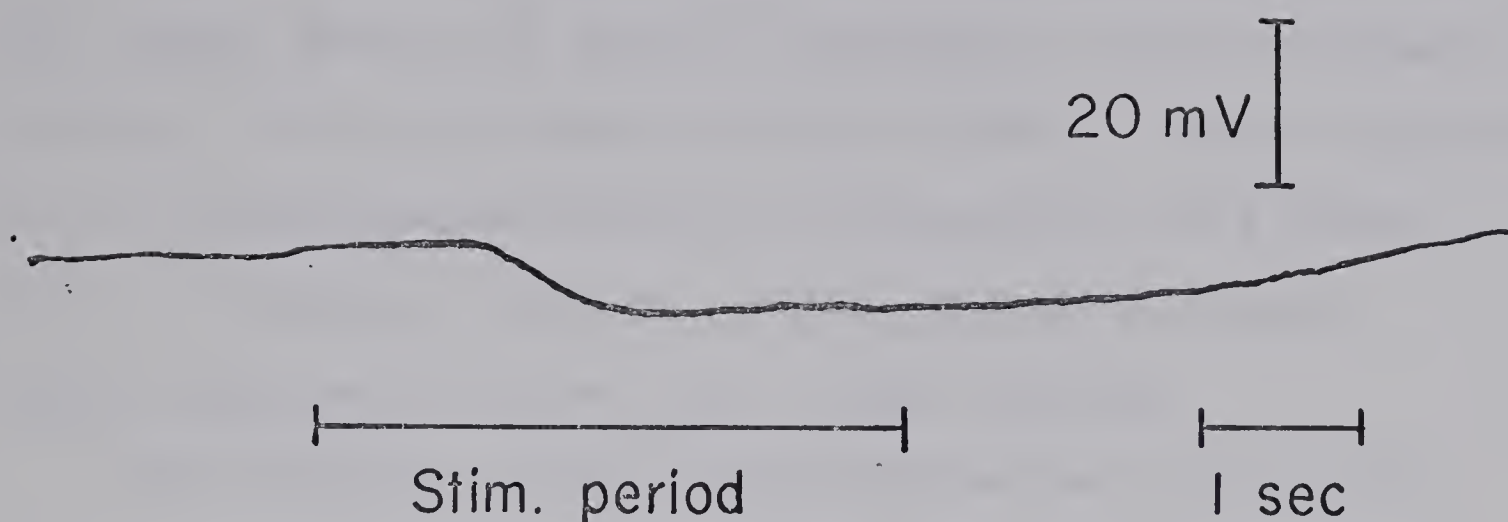


Figure 21. Secretory cell response to sympathetic nerve stimulation. The sympathetic nerve was stimulated at a frequency of 10 stimuli per second.



Figure 22. Secretory cell response to sympathetic nerve stimulation. The microelectrode is out of the cell at the end of the trace.



The electrophysiological results obtained as part of this thesis agree with results obtained by previous workers, Lundberg (1955) and Creed and Wilson (1969). Chorda tympani nerve induced hyperpolarization of secretory cells occurs after a latency of 300 msec., while sympathetic nerve induced hyperpolarization has a longer latency.

The mechanism for the hyperpolarization response has not been established at the present time. An ionic mechanism for the hyperpolarization response has recently been suggested by Petersen and Poulsen (1967, 1968) and Petersen (1970). Petersen (1970) concludes that the hyperpolarization is probably due to an outward potassium current partially short-circuited by an inward sodium current. The potassium equilibrium potential across the basal acinar cell membrane is greater than the maximum hyperpolarization of the cell while the sodium potential is less than the resting membrane potential. Petersen showed that the magnitude of the hyperpolarization increased during perfusion of the gland with potassium-free solution (NaCl) and during perfusion with large non-permeating ions instead of sodium (TEA Cl).

#### 5.4B Blood Flow

Blood flow was measured using the forced convection flow meter. A vasodilatation response of the submaxillary gland to chorda tympani nerve stimulation is shown in



Figure 23. The vasodilatation is quick in onset.

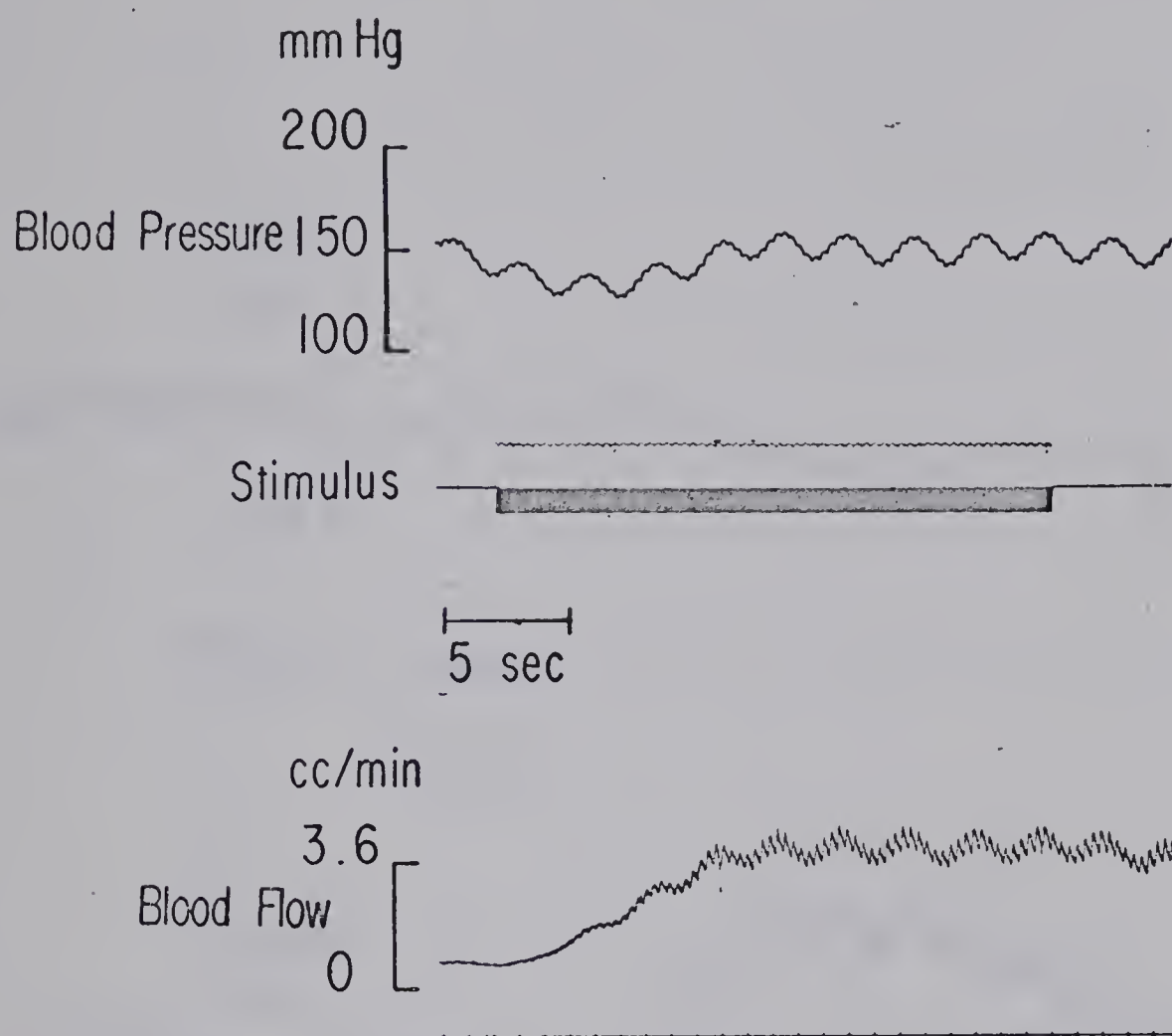


Figure 23. Vasodilatation in the cat submaxillary gland as a result of chorda tympani nerve stimulation. Also shown is the stimulus and blood pressure.

Figure 24 shows the response of submaxillary gland blood flow to sympathetic nerve stimulation. The blood flow decreases after a latency of about 1 sec. and after the stimulation has stopped the blood flow increases to a value greater than the basal flow rate. In a few cases, although this is not evident in Figure 24, this after-





dilatation exceeds the vasodilatation produced by optimal stimulation of the chorda tympani. This after-dilatation was first described by Carlson (1907).

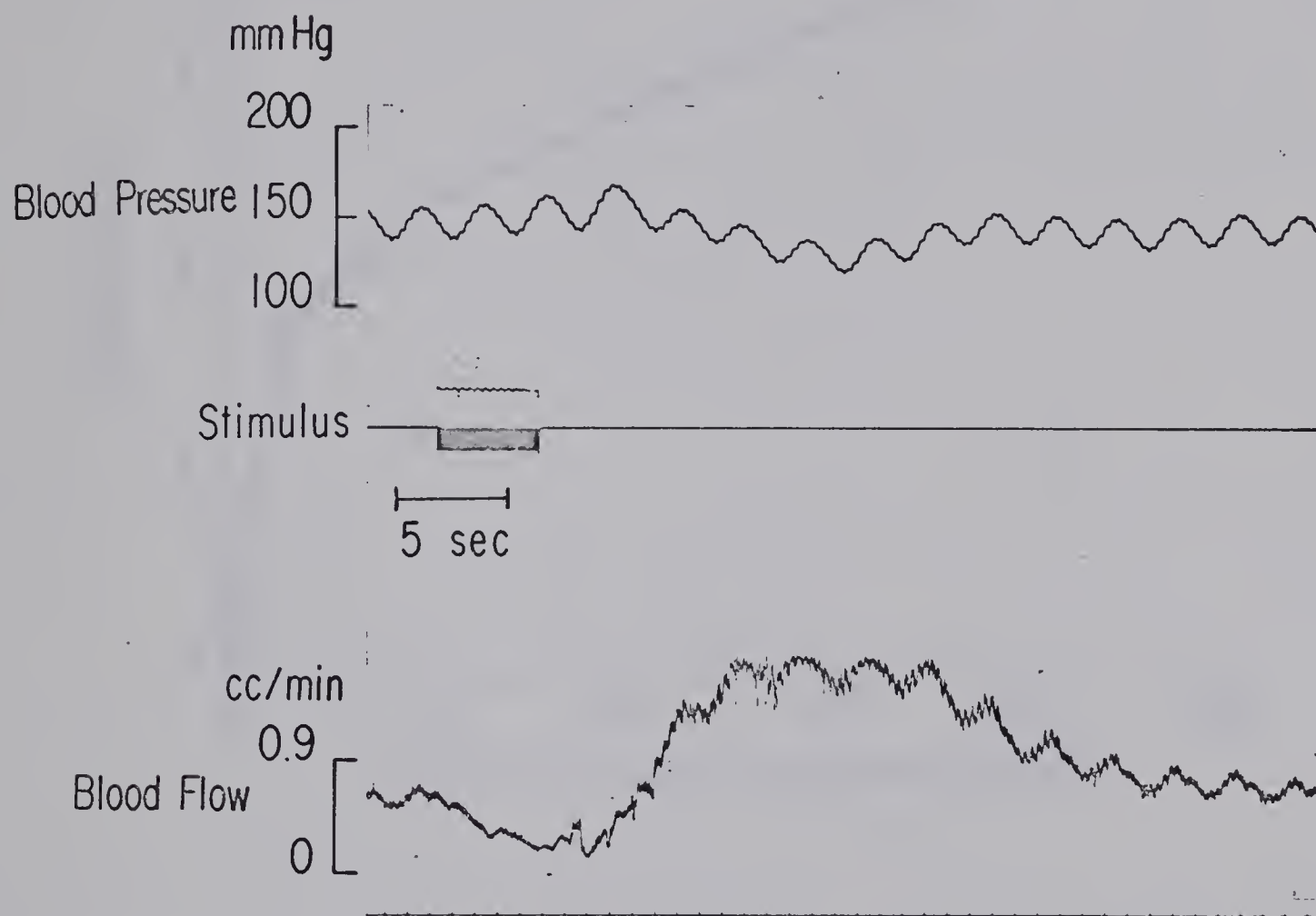


Figure 24. Vasoconstriction in the cat submaxillary gland as a result of sympathetic nerve stimulation.

In some of the preparations, stimulation of the chorda tympani nerve with a single stimulus or short pulse trains elicited vasodilatation. Figure 25 shows the response of one such gland where volume increase (integral of flow rate) is plotted versus the number of stimuli. This shows the



high sensitivity of the measurement system.

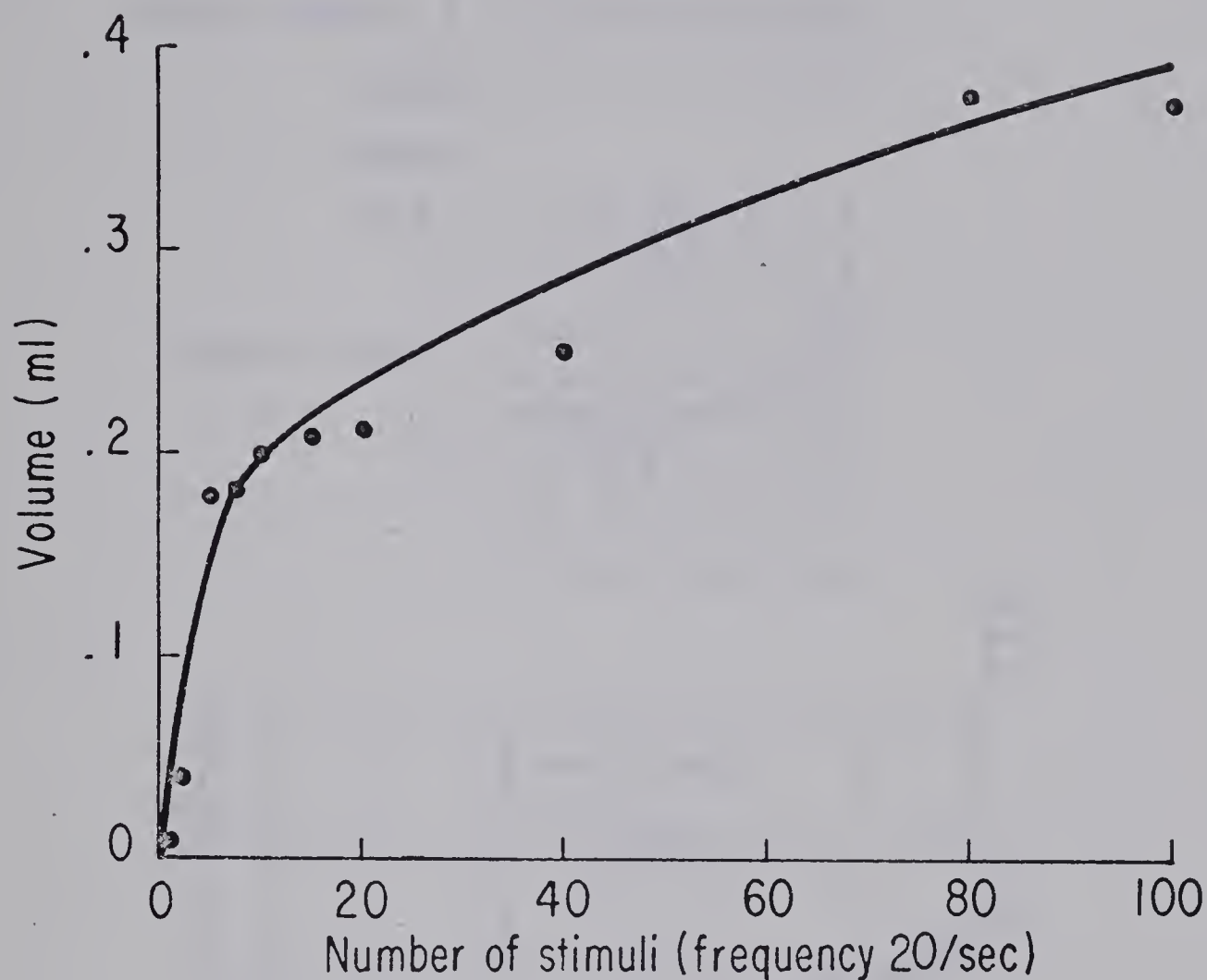


Figure 25. Volume increase versus number of stimuli (integral of flow rate). The interval between stimuli was 50 msec. (20/sec.) in cases of more than one pulse.

Latency measurements were made on several different preparations. The latency was taken as the time between the beginning of nerve stimulation and the onset of vasodilatation. One of the shortest latencies is shown in Figure 26. Figure 26A shows the vasodilatation response due to a 3 second period of chorda tympani nerve stimulation. Figure 26B



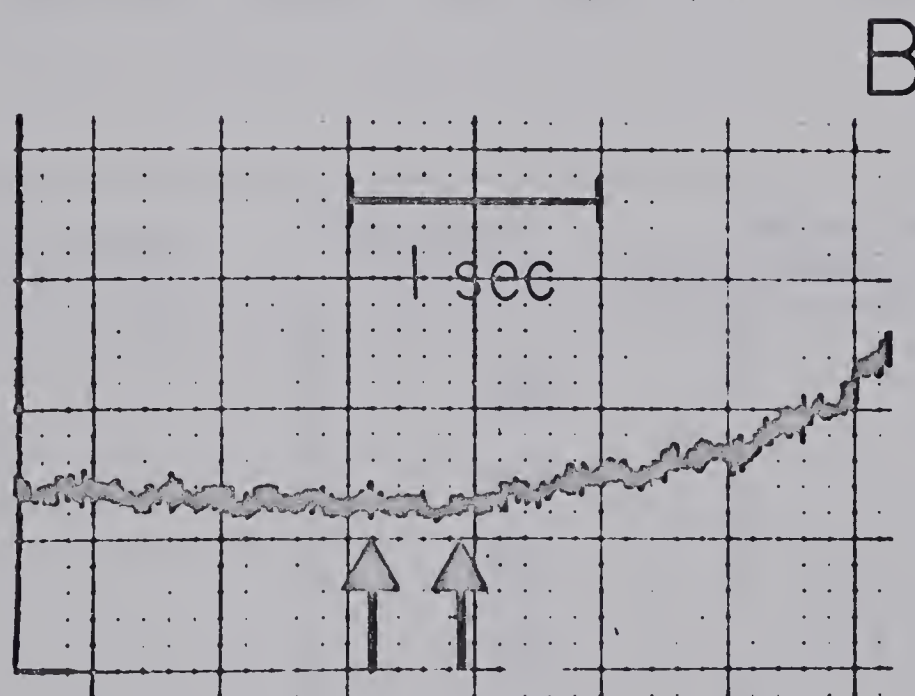
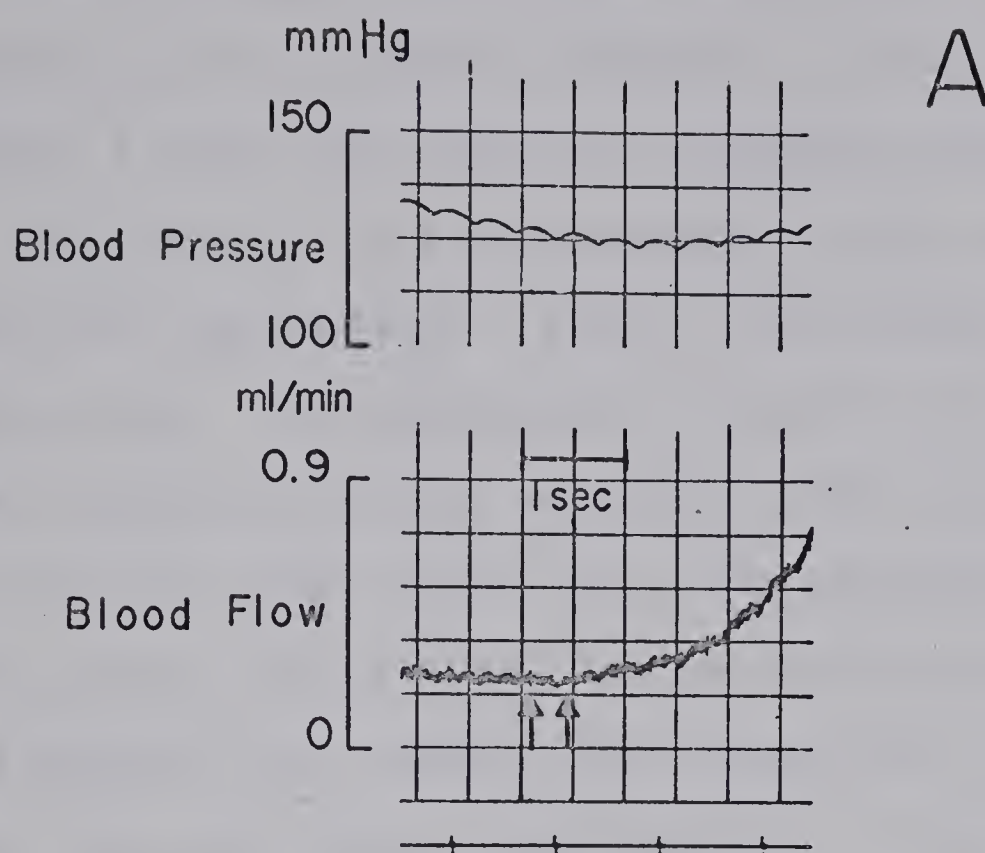


Figure 26. Vasodilatation in the cat submaxillary as a result of a 3 second period of chorda tympani nerve stimulation.

Figure 26A. Blood pressure and blood flow. The first arrow indicates the beginning of stimulation, while the second arrow indicates the onset of vasodilatation.

Figure 26B. Enlarged portion of the vasodilatation response shown in A.



shows an enlarged portion of the vasodilator response. The latency in this case is 350 msec. or less.

Table 1 gives the shortest latencies measured in five cats. The means of four measurements in the same animal are given for experiments 3 and 4. The standard deviations are also given. In experiments 1, 2 and 5 the values given are the shortest latencies measured in the experiments. In these particular experiments only one reasonable measurement of latency was obtained due to experimental difficulties. Other latencies were usually much longer due to a decrease in basal flow and general deterioration of the experimental animal.

Experiment	Shortest Latency (msec.)	Mean Latency (msec.) $\pm$ SD
1	800	--
2	900	--
3	550	560 $\pm$ 100
4	350	340 $\pm$ 55
5	650	--

Table 1. Shortest latencies between chorda tympani nerve stimulation and onset of vasodilatation.

The onset of vasodilatation was taken as the point at which a change in slope occurred. Straight lines were





fitted to the blood flow record (first order approximation). The first straight line was fitted to the basal flow portion of the blood flow record and the second was fitted to the initial phase of the vasodilatation response. The intersection of these straight lines was taken as the onset of vasodilatation. The latencies obtained in this manner were compared to those obtained by an unbiased observer from the same record and were usually larger by 25 msec. or were of the same magnitude. In experiment 4 latencies were obtained which were shorter than shown in Figure 26. The shortest latency obtained in experiment 4 was 250 msec., but the vasodilatation was paralleled by an increase in blood pressure.

The latencies in Table 1 show a wide variation. However, the most important one is that in experiment 4 since its magnitude is the same as the latency between chorda tympani nerve stimulation and hyperpolarization of the secretory cells. This shows that vasodilatation is not secondary to secretion. Although the latencies in other experiments exceed the shortest latency, this does not invalidate the above conclusion.

Some of the variation in latencies shown in Table 1 is probably due to mechanical factors. For example, since



veins are collapsible structures, a change in blood flow at one point does not produce an instantaneous change at a distal point. In the experimental set-up all of the veins, except the submaxillary vein supplying the external jugular are tied off and the vessel is in a partially collapsed state. In vitro measurement on the external jugular vein showed that the time delay between a small pressure change in the submaxillary vein and flow changes at the probe (3 cm. away) was of the order of 50 msec. or more. The basal flow was similar to that shown in Figure 23 and the blood vessel was from the experimental animal listed as cat 3 in Table 1. This time delay measurement is shown in Figure 27.

#### 5.4C Experiments With Bradykinin Potentiating Factor

The synthetic bradykinin potentiating peptide developed by Ferreira, Bartelt and Greene (1970) has the same spectrum of properties as previously reported by Ferreira (1965) for the Bradykinin Potentiating Factor (B.P.F.). The B.P.F. is a peptide fraction from Bothrops jararaca venom which inhibits the enzymes that normally inactivate bradykinin and the enzyme responsible for the conversion of angiotensin I to angiotensin II. The peptide potentiates the depressor effects of bradykinin on blood pressure and also potentiates the effect of bradykinin on capillary permeability.



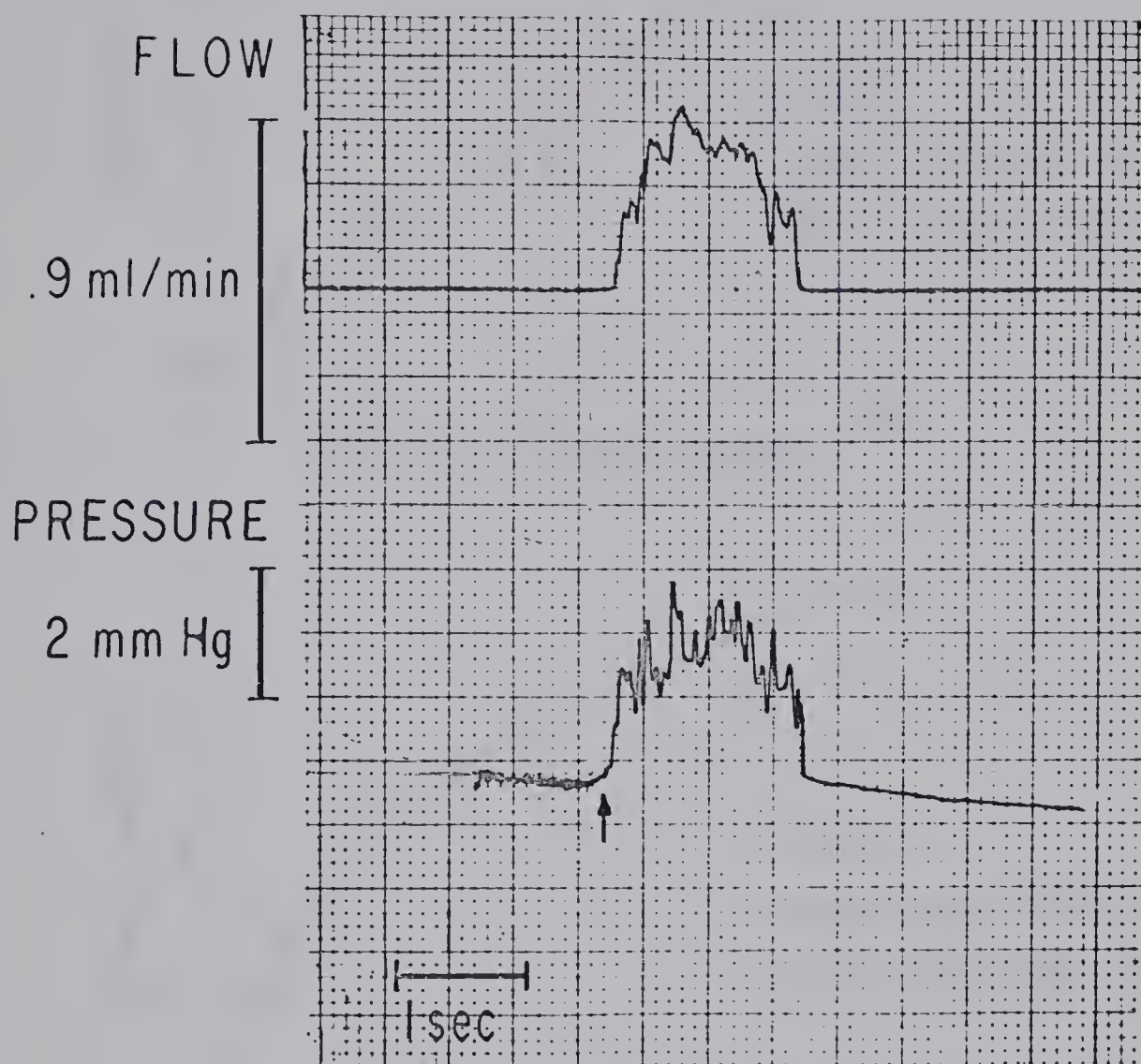


Figure 27. Time delay between pressure change in the submaxillary vein and the resultant flow change in the probe (3 cm. away). The arrow shows the beginning of the pressure pulse.

This experiment is similar to Skinner and Websters' work with carboxypeptidase B. Skinner and Webster (1968) reported that chorda tympani nerve induced vasodilatation was unaffected in cats pretreated with carboxypeptidase B, an inactivator of bradykinin. Skinner and Webster suggested





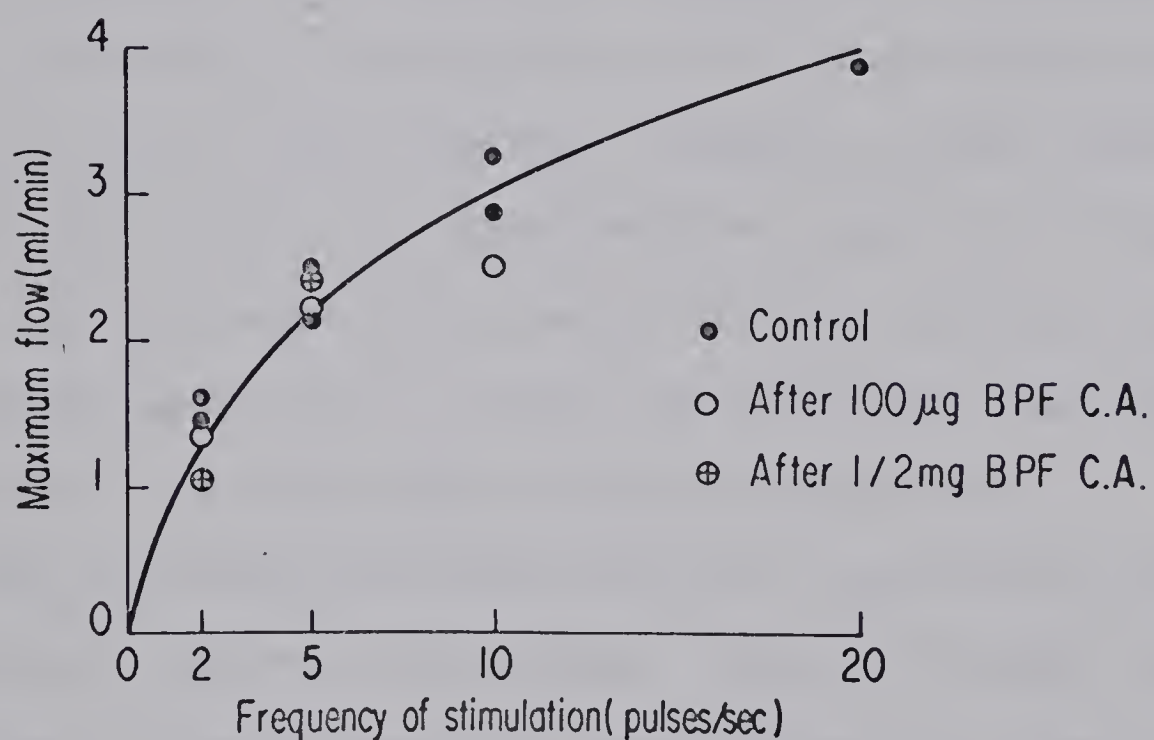
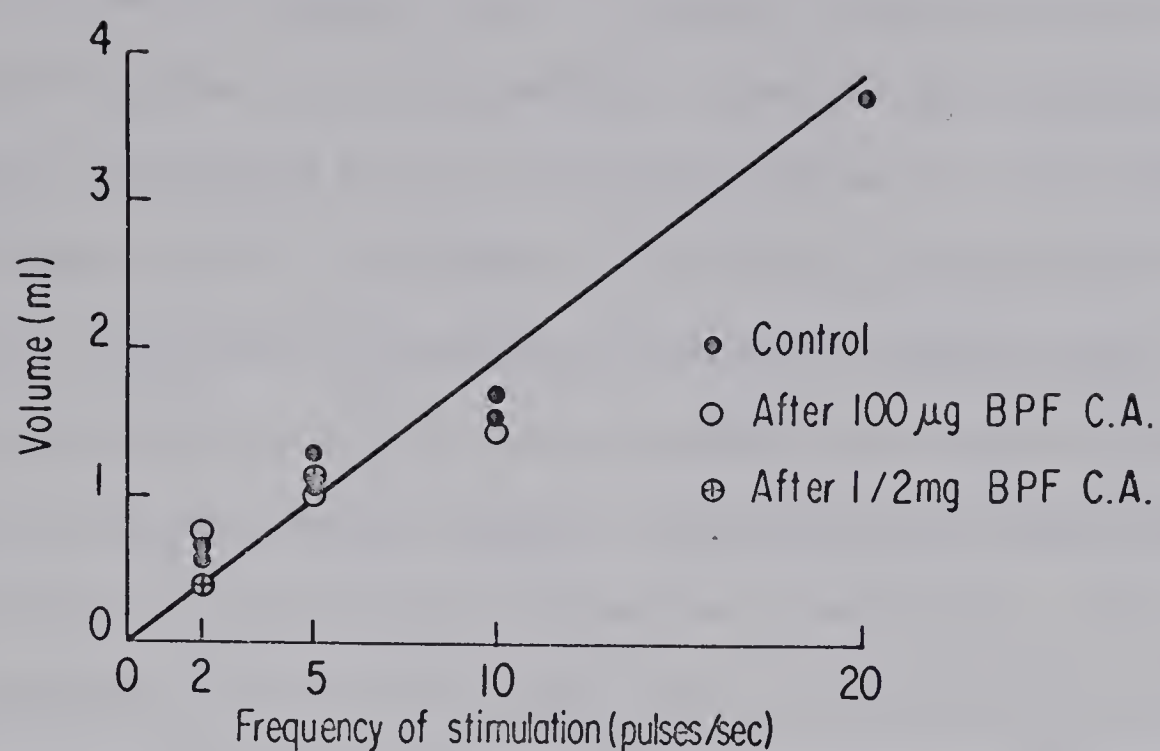


Figure 28. Vasodilatation in the submaxillary gland as a result of chorda tympani nerve stimulation before and after B.P.F. B.P.F. was injected close-arterially. The first curve is a plot of volume increase versus frequency of stimulation. The second curve is a plot of maximum flow rate versus frequency of stimulation. The duration of stimulation was 30 seconds in all cases.





that if chorda tympani nerve induced vasodilatation was mediated by the kallikrein-kinin system, the response in animals pretreated with carboxypeptidase B, would be diminished if not abolished. A similar experiment is one in which the kallikrein-kinin system is potentiated. A substance such as B.P.F. which potentiates bradykinin, should enhance chorda tympani nerve induced vasodilatation if the kallikrein-kinin system is a mediator. The B.P.F. was injected close-arterially and no potentiation of chorda tympani nerve induced vasodilation was observed. Figure 28 shows a comparison of the two responses before and after B.P.F. was injected. However, these results are inconclusive since no vasodilatation could be elicited with 2 ug of bradykinin injected close-arterially before the injection of B.P.F. Only one experiment was completed because of the difficulty in obtaining B.P.F.

The potentiating effect of B.P.F. was easily demonstrated on the guinea pig ileum. Figure 29 shows the response of the guinea pig ileum before and after B.P.F.



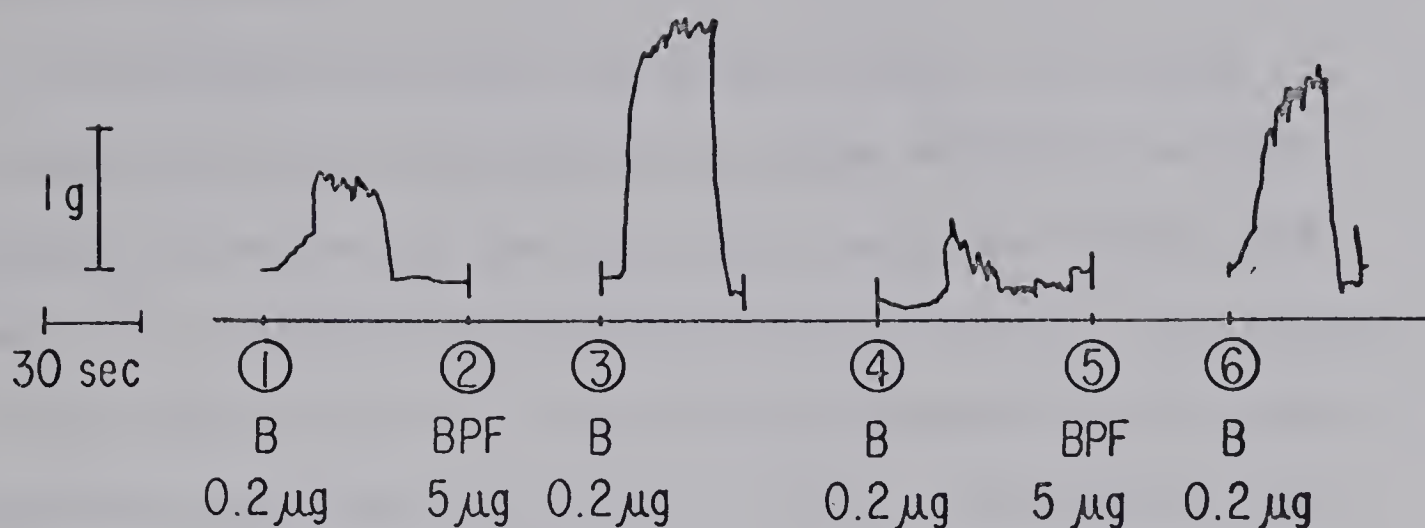


Figure 29. Potentiating effect of B.P.F. on the bradykinin response of the guinea pig ileum. At 1, 0.2 ug of bradykinin was added into the bath. After the response had returned to a control level, 5 ug of B.P.F. was added at 2. Thirty seconds later at 3, 0.2 ug of bradykinin was added. At 4, 5 and 6 the sequence was repeated.



## 5.5 Discussion

According to Creed and Wilson (1969), the mean latency between parasympathetic nerve stimulation and hyperpolarization of the secretory cell, was about 300 msec. at a stimulation frequency of 20 stimuli per second. Of this, about 60 msec. could be accounted for by nerve conduction and ganglionic delay, while the greater part of this latency was attributed to the diffusion of the transmitter and initiation of hyperpolarization. The magnitude of the latency between chorda tympani nerve stimulation and hyperpolarization measured in this work was the same magnitude as that reported by Creed and Wilson.

The shortest measured latency between chorda tympani nerve stimulation and vasodilatation was between 300 and 350 msec. This latency is shorter since a transport delay exists as was shown in Figure 27. The actual magnitude of the transport delay is difficult to estimate. However, it seems likely that vasodilatation occurs before, or is simultaneous with, hyperpolarization. In either case, it seems unlikely that chorda tympani nerve induced vasodilatation is mediated by the kallikrein-kinin system or that it is secondary to secretion.

Even in those experiments where the latency is longer (i.e. 900 msec.), there does not seem to be time for the kallikrein to act as a mediator of chorda tympani nerve induced vasodilatation. This mechanism involves the release





of kallikrein from secretory cells, its passage into the interstitial fluid, its action on an  $\alpha_2$ -globulin substrate to release kallidin, and the vasodilator action of the kinin on the smooth muscle in the walls of the blood vessels. Although very little is known about the enzyme kinetics involved, in vitro experiments on the guinea pig ileum indicate a slow system.

This view is also held by Gautvik (1970a, b, c). Using a drop counter to measure the venous outflow from the cat submaxillary gland, he estimated the latency between chorda tympani nerve stimulation and onset of vasodilatation to be between 1 and 6 seconds. Gautvik concluded that it seemed unlikely that a mechanism involving release of an enzyme from gland cells, and its successive participation in chemical reaction (s), could be effective within a few seconds. The work in this thesis has shown conclusively that the latency is about 350 msec. or less.

Gautvik suggests that vasodilatation is initiated by nerve fibers, but that the kallikrein-kinin system is also a part of vasodilator response. Thus a nervous mechanism ensures an immediated increase in blood flow, while the kallikrein-kinin system is responsible for the maintenance of blood flow in accordance with metabolic needs. This type of two-phase system was first suggested by Barcroft (1914). His hypothesis was that under normal circumstances dilation may be instituted by dilator fibers and maintained by metabolic products. This was strongly supported by





combined vascular and secretory studies done by Holzlöhner and Hoffman (1931).

A comparison of the latencies which exist when the sympathetic nerve is stimulated, shows that vasoconstriction has a latency of about 1000 msec. and hyperpolarization has a latency of about 650 msec. When the parasympathetic nerve to the gland is stimulated, hyperpolarization of the secretory cells and vasodilatation occur after a latency which is about 300 msec. Since it is generally accepted that vasoconstriction is directly controlled by the sympathetic nerve, the shortness of the latency which precedes parasympathetic vasodilatation, lends support to the theory that parasympathetic vasodilator nerve fibers exist.

Preliminary experiments with B.P.F. showed no potentiation of chorda tympani nerve induced vasodilatation, but since close-arterial injection of bradykinin did not elicit vasodilatation, the results are inconclusive.

The results shown in this work would tend to support the theory that chorda tympani nerve induced vasodilatation is mediated by a direct nervous mechanism. This mechanism would involve the release of a transmitter such as acetylcholine. However, no evidence is available as to the nature of the transmitter or the mechanism involved, nor is it evident at the present time why the vasodilatation is not blocked by moderate doses of atropine if the transmitter is acetylcholine as is suggested by Schachter



and Beilenson (1968).

In order to explain parasympathetic induced vasodilatation after atropine, Gautvik (1970c) has postulated two neuro-effector mechanisms. One mechanism controls the release of kallikrein from secretory cells and the other one acts directly on the resistance vessels. Both postulated mechanisms are atropine resistant.

The results of this thesis quite conclusively rule out the possibility that the kallikrein-kinin system plays a role in the initial phase of chorda tympani nerve induced vasodilatation, but they do not reveal anything about the possibility that maintained vasodilatation is mediated by a metabolic product, as is suggested by Barcroft (1914), Holzlöhner and Hoffman (1931) and Gautvik (1970a, b, c).

The only method which would conclusively show the existence of parasympathetic vasodilator fibers is electrophysiological recording from smooth muscle cells in the walls of blood vessels. If successful, the latency of the electrical response of vascular smooth muscle would give a final answer to the question of whether parasympathetic vasodilator nerve fibers exist or not in the submaxillary gland of the cat.

Some work which applies to this type of problem has been reported. Funaki (1958, 1960, 1961) recorded resting membrane potentials and action potentials in smooth muscle cells in the blood vessels of the tongue and abdominal skin of the frog. Funaki (1961) reported resting membrane



potentials of about 40 mV and spike potentials of 60 mV. in magnitude and 200 msec. in duration. He also reported that the resting potential of endothelial cells was about 47 mV with no action potentials present.

Steedman (1966) has also reported recent work in this area. He used as one of his preparations the smooth muscle cells in the walls of small arteries in the tongue and skin of the lateral abdomen in the frog. Another preparation used by Steedman was the smooth muscle cells in the walls of small arteries and arterioles of the rat mesenteric circulation. He reported resting potentials with mean values of 64.7 mV in the frog tongue and 43.6 mV in the skin of the lateral abdomen. Action potentials of up to 60 mV in amplitude and 100 - 200 msec. in duration could be elicited by electrical stimulation of the blood vessel wall in a position near the recording electrode. This result was reported in the frog tongue blood vessels.

In the work by Steedman on the rat mesenteric circulation, intracellular potentials varied continually with a maximum polarization of 39.4 mV. The slow waves had periods varying from 4.7 - 7.8 seconds. Spike potentials were recorded with amplitudes of up to 35 mV and durations varying from 40 - 50 msec. Steedman also reported that interference with the autonomic nervous system, chemically or by denervation, affected the amplitude of slow waves but not the frequency and occurrence of the action potentials. Stimulation of the smooth muscle cells by electri-







cal stimulation of the splanchnic nerves and local application of adrenalin, noradrenalin and vasopressin increased the frequency of action potentials and the amplitude of the slow waves, while acetylcholine and removal of the nerve supply depressed the electrical activity.

The work of Funaki and Steedman indicates that if nerve activity could be related to smooth muscle activity (blood vessels) in the salivary gland, the question of whether parasympathetic vasodilator nerves exist would be answered.

This work is in progress at the present time with very little success. Due to the small size of the muscle cells and difficulties with the connective tissue the measurements are proving difficult.

## 5.6 Conclusions

(1) Chorda tympani nerve induced vasodilatation occurs before, or at the same time as, hyperpolarization of secretory cells.

(2) The initial phase of chorda tympani nerve induced vasodilatation is not mediated by the kallikrein-kinin system.



## Bibliography

Barcroft, J. (1914). "The respiratory function of the blood". Cambridge University Press. London.

Barcroft, J. and Müller, F. (1912). The relation of blood flow to metabolism in the submaxillary gland. *J. Physiol.* 44, 259.

Barcroft, J. and Piper, H. (1912). The gaseous metabolism of the submaxillary gland with reference especially to the effect of adrenalin and the time relation of the stimulus to the oxidation process. *J. Physiol.* 44, 359.

Bayliss, W.M. (1923). "The Vasomotor System". Longmans, Green and Co., New York.

Bayliss, W.M. and Bradford, J.R. (1886). The electrical phenomena accompanying the process of secretion in the salivary glands of the dog and cat. *Proc. Roy. Soc.* 40, 203.

Beilenson, S., Schachter, M. and Smaje, L.H. (1965). Kallikrein in the submaxillary gland of the cat. *J. Physiol.* 177, 61P.

Beilenson, S., Schachter, M. and Smaje, L.H. (1968). Secretion of kallikrein and its role in vasodilatation in the submaxillary gland. *J. Physiol.* 199, 303.



- Bernard, C. (1853). De l'influence de deux ordres de nerfs qui determinent les variations de couleur du sang veineux dans les organes glandulaires. *C.R. Acad. Sci.* 47, 245.
- Bhoola, K.D., Morley, J., Schachter, M. and Smaje, L.H. (1965). Vasodilatation in the submaxillary gland of the cat. *J. Physiol.* 179, 172.
- Boussinesq, M.J. (1905). Calcul du pouvoir refroidissant des courants fluides. *J. de Math.* 1, 285.
- Bradford, J.R. (1887). The electrical phenomena accompanying the excitation of so called secretory and trophic nerve fibres in the salivary glands of the dog and cat. *J. Physiol.* 8, 86.
- Carlson, A.J. (1907). Vasodilator fibres to the submaxillary gland in the cervical sympathetic of the cat. *Amer. J. Physiol.* 19, 408.
- Corrsin, S. (1963). Turbulence: Experimental methods. In *Encyclopedia of Physics*, ed. Flügge, S., p. 524. Springer-Verlag, Berlin VIII/2.
- Creed, K.E. and Wilson, J.A.F. (1969). The latency of response of secretory acinar cells to nerve stimulation in the submandibular gland of the cat. *Aust. J. Exp. Biol. Med. Sci.* 47, 135.
- Davey, M.J., Davies, R.F., Reinert, H. and Scholfield, P. (1965). Effects of adrenergic-neurone blocking agents on the submaxillary gland of the cat. *Nature.* 205, 673.





- Ferreira, S.H. (1965). A bradykinin-potentiating factor (BPF) present in the venom of Bothrops jararaca. *Brit. J. Pharmacol. Chemother.* 24, 163.
- Ferreira, S.H., Bartelt, D.C. and Greene, L.J. (1970). Isolation of bradykinin potentiating peptides from Bothrops jararaca. *Biochem.* In Press.
- Funaki, S. (1958). Studies on membrane potentials of vascular smooth muscle with intracellular micro-electrodes. *Proc. Japan Acad.* 34, 534.
- Funaki, S. (1960). Electrical activity of single vascular smooth muscle fibres. In *Electrical Activity of Single Cells*. Tokyo: Igakushoin; Hongo.
- Funaki, S. (1961). Spontaneous spike-discharge of vascular smooth muscle. *Nature, Lond.* 191, 1102.
- Garrett, J.R. (1966c). The innervation of salivary glands. III. The effect of certain experimental procedures on the cholinesterase-positive nerves in glands of the cat. *J. roy. micr. Soc.* 86, 1.
- Garrett, J.R. (1966d). The innervation of salivary glands. IV. The effects of certain experimental procedures on the ultrastructure of nerves in glands of the cat. *J. roy. micr. Soc.* 86, 15.
- Gautvik, K. (1970a). Studies on kinin formation in functional vasodilatation of the submandibular salivary gland in cats. *Acta physiol. scand.* 79, 174.





- Gautvik, K. (1970b). The interaction of two different vasodilator mechanisms in the chorda-tympani activated submandibular salivary gland. *Acta physiol. scand.* 79, 188.
- Gautvik, K. (1970c). Parasympathetic neuro-effector transmission and functional vasodilatation in the submandibular salivary gland of cats. *Acta physiol. scand.* 79, 204.
- Grahn, A.R., Paul, M.H. and Wessel, H.U. (1968). Design and evaluation of a new linear thermistor velocity probe. *J. Appl. Physiol.* 24, 236.
- Grahn, A.r., Paul, M.H. and Wessel, H.U. (1969). A new direction sensitive probe for catheter-tip thermal velocity measurements. *J. Appl. Physiol.* 27, 407.
- Heidenhain, R. (1872). Ueber die Wirkung einiger Gifte auf die Nerven der glandula submaxillaris. *Arch. ges. Physiol.* 5, 309.
- Hilton, S.M. (1960). Plasma kinin and blood flow. In *Polypeptides which affect smooth muscles and blood vessels*, ed. Schachter, M., p 326. Pergamon Press, London.
- Hilton, S.M. (1962). Local mechanisms regulating peripheral blood flow. *Physiol. Rev. Supp.* 5, 265.
- Hilton, S.M. (1963). A discussion of the evidence for kinins as the agents of vasodilator reactions. *Anns. N.Y. Acad.* 104, 275.



- Hilton, S.M. (1970). The physiological role of glandular kallikreins. In *Handbook exp Pharmacol.* p 389.
- Hilton, S.M. and Lewis, G.P. (1955a). The cause of the vasodilatation accompanying activity in the submandibular gland. *J. Physiol.* 128, 235.
- Hilton, S.M. and Lewis, G.P. (1955b). The mechanism of the functional hyperaemia in the submandibular salivary gland. *J. Physiol.* 129, 253.
- Hilton, S.M. and Lewis, G. P. (1956). The relationship between glandular activity, bradykinin formation and functional vasodilatation in the submandibular salivary gland. *J. Physiol.* 134, 471.
- Hilton, S.M. and Lewis, G.P. (1957). Functional vasodilatation in the submandibular salivary gland. *Brit. Med. Bull.* 13, 189.
- Hilton, S.M. and Lewis, G.P. (1958). Vasodilatation in the tongue and its relationship to plasma kinin formation. *J. Physiol.* 144, 532.
- Hilton, S.M. and Torres, S. H. (1967). Bradykinin and functional vasodilatation in the submandibular salivary gland in the cat. *J. Physiol.* 189, 69P.
- Hinze, J.O. (1959). "Turbulence". Mc Graw-Hill., New York-Toronto-London.
- Holzlohner, E. and Hoffman, F. (1931). Die Drüsentätigkeit bei Nervenreizung. II. Die Beziehungen zwischen Blutstrom und Sekretstrom der Glandula submaxillaris bei Chordareizung. *Z. Biol.* 91, 552.



- James, G.W., Paul, M.H. and Wessel, H.U. (1966). Thermal dilution: instrumentation with thermistors. *J. Appl. Physiol.* 21, 1131.
- Janssen, J.M.L., Ensing, L. and Van Erp, J.B. (1959). A constant-temperature operated hot wire anemometer. *Proc. I.R.E.* 47, 555.
- Katsura, S., Weiss, R., Baker, D. and Rushmer, R.F. (1959). Isothermal blood flow velocity probe. *I.R.E. Trans. Med. Elec.* 6, 283.
- King, L.V. (1914). On the convection of heat from small cylinders in a stream of fluid: determination of the convection constants of small platinum wires with application to hot wire anemometry. *Phil. Trans. Roy. Soc. Lond.* A214, 373.
- Kudryashev, L.I. and Smirnov, A.A. (1965). Estimation of influence of thermal unsteady state on convective heat transfer coefficient for spherical bodies in flow at small Reynold's numbers. *Proc. of the Second All-Soviet Union Conference of Heat and Mass Transfer.* 1, 343.
- Kuntz, A. and Richins, C.A. (1946). Components and distribution of the nerves of the parotid and submandibular glands. *J. comp. neurol.* 85, 21.
- Landau, L.D. and Lefshitz, E.M. (1959). "Fluid Mechanics". Pergamon Press, London.
- Lewis, G.P. (1960). Active polypeptides derived from plasma proteins. *Physiol. Rev.* 40, 647.





- Lewis, G.P. (1962). Pharmacological actions and function of bradykinin. *Biochem. Pharmacol.* 10, 29.
- Lewis, G.P. (1963). Pharmacological actions of bradykinin and its role in physiological and pathological reactions. *Ann. N.Y. Acad. Sci.* 104, 236.
- Lundberg, A. (1955). The electrophysiology of the submaxillary gland of the cat. *Acta physiol. scand.* 35, 1.
- Lundberg, A. (1957a). Secretory potentials in the sublingual gland of the cat. *Acta physiol. scand.* 40, 21.
- Lundberg, A. (1957b). The mechanism of establishment of secretory potentials in sublingual gland cells. *Acta physiol. scand.* 40, 35.
- Lundberg, A. (1957c). Anionic dependence of secretion and secretory potentials in the perfused sublingual gland. *Acta physiol. scand.* 40, 101.
- Lundberg, A. (1958). Electrophysiology of salivary glands. *Physiol. Rev.* 38, 21.
- Ludwig, C. (1851). Neue Versuche über die Beihilfe der Nerven zur Speichelabsonderung. *Z. rat. Med. N.F.* 1, 255.
- Morley, J., Schachter, M. and Smaje, L.H. (1966). Vaso-dilatation in the submaxillary gland of the rabbit. *J. Physiol.* 187, 595.



- Petersen, O.H. (1970). Transmembrane secretory potentials in the cat submandibular gland during perfusion with potassium-free and low sodium Locke solutions. *Experientia*. 26, 612.
- Petersen, O.H. and Poulsen, J.H. (1967). The effects of varying the extracellular potassium concentrations on the secretory rate and on resting and secretory potentials in the perfused cat submandibular gland. *Acta physiol. scand.* 70, 243.
- Petersen, O.H. and Poulsen, J.H. (1968). Secretory potentials, potassium transport and secretion in the cat submandibular gland during perfusion with sulfate Locke's solution. *Experientia*. 24, 919.
- Prasad, K. and MacLeod, D.P. (1966). Effect of hemicholinium no. 3 on the response of frog rectus abdominus muscle to acetylcholine. *Can. J. Physiol. Pharmacol.* 44, 179.
- Rasmussen, R.A. (1962). Application of thermistors to measurements in moving fluids. *Rev. Sci. Instr.* 33, 38.
- Richardson, K.C. (1964). The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscle. *Am. J. Anat.* 114, 173.



- Schachter, M. and Beilenson, S. (1967). Kallikrein and vasodilatation in the submaxillary gland. *Gastroenterology*. 52, 401.
- Schachter, M. and Beilenson, S. (1968). Mediator of vasodilatation in the submaxillary gland. *Federation Proc.* 27, 73.
- Skinner, N.S. and Webster, M.E. (1968). Submaxillary gland blood flow: the role of kinins and beta-adrenergic receptors. *Federation Proc.* 27, 76.
- Snell, R.S. and Garrett, J.R. (1958). The effect of post-ganglionic sympathectomy on the histochemical appearances of cholinesterase in the nerves supplying the submandibular and sublingual salivary glands of the rat. *Zeitschrift für Zellforschung*. 48, 201.
- Steedman, W.M. (1966). Micro-electrode studies on mammalian vascular muscle. *J. Physiol.* 186, 382.
- Strömblad, B.C.R. and Dresel, P.E. (1963). Experiments on atropine-resistant vasodilatation in salivary glands. *Can. J. Biochem. Physiol.* 41, 519.
- Terroux, K.G., Sekelj, P. and Burgen, A.S.V. (1959). Oxygen consumption and blood flow in the submaxillary gland of the dog. *Can. J. Biochem. Physiol.* 37, 5.
- Ungar, G. and Parrot, J.L. (1936). Sur la présence de la callicréine dans la salive, et la possibilité de son intervention dans la transmission chimique de l'influx nerveux. *C. R. Soc. Biol. Paris*. 122, 1052.



Werle, E. and Von Roden, P. (1936). Über das Vorkommen von Kallikrein in den Speicheldrüsen und im Mundspeichel. *Biochem. Z.* 286, 213.

Ziegler, M. (1934). "The Construction of a Hot-Wire Anemometer with Linear Scale and Negligible Lag".  
Laboratorium voor Aerodynamica et Hydrodynamica der Technische Hogeschool te Delft, in Verhandelingen der Kon. Ned. Akadamie van Wetenschappen te Amsterdam, Afdeling Natuurkunde, Mededeling 29.





The flow meter, as described in Chapters 2, 3 and 4, was more than adequate for use in measuring the venous outflow from the submaxillary gland.

(1) Flow sensitivity--measures less than 0.05 ml./min. (0.08 cm./sec. in 1.6 mm. probe).

(2) Bandwidth at zero velocity estimated 125 Hz to increasing and decreasing velocity (increases with increasing velocity).

(3) Noise--less than 0.5 mV r.m.s. referred to the input of the squaring circuit (zero velocity).

--0.6 mV r.m.s. referred to the input of the squaring circuit (velocity 0.4 cm./sec.).

Sensitivity is a factor of 100 times greater than that achieved by electromagnetic or ultrasonic techniques. The bandwidth is much higher than that achieved with the drop counter or thermistors and thermocouples operated in the constant current mode.



## A2.1 General Scheme

A block diagram showing the arrangement of the forced convection flow meter is shown in Figure A-1. This is the same as is shown in Figure 7.

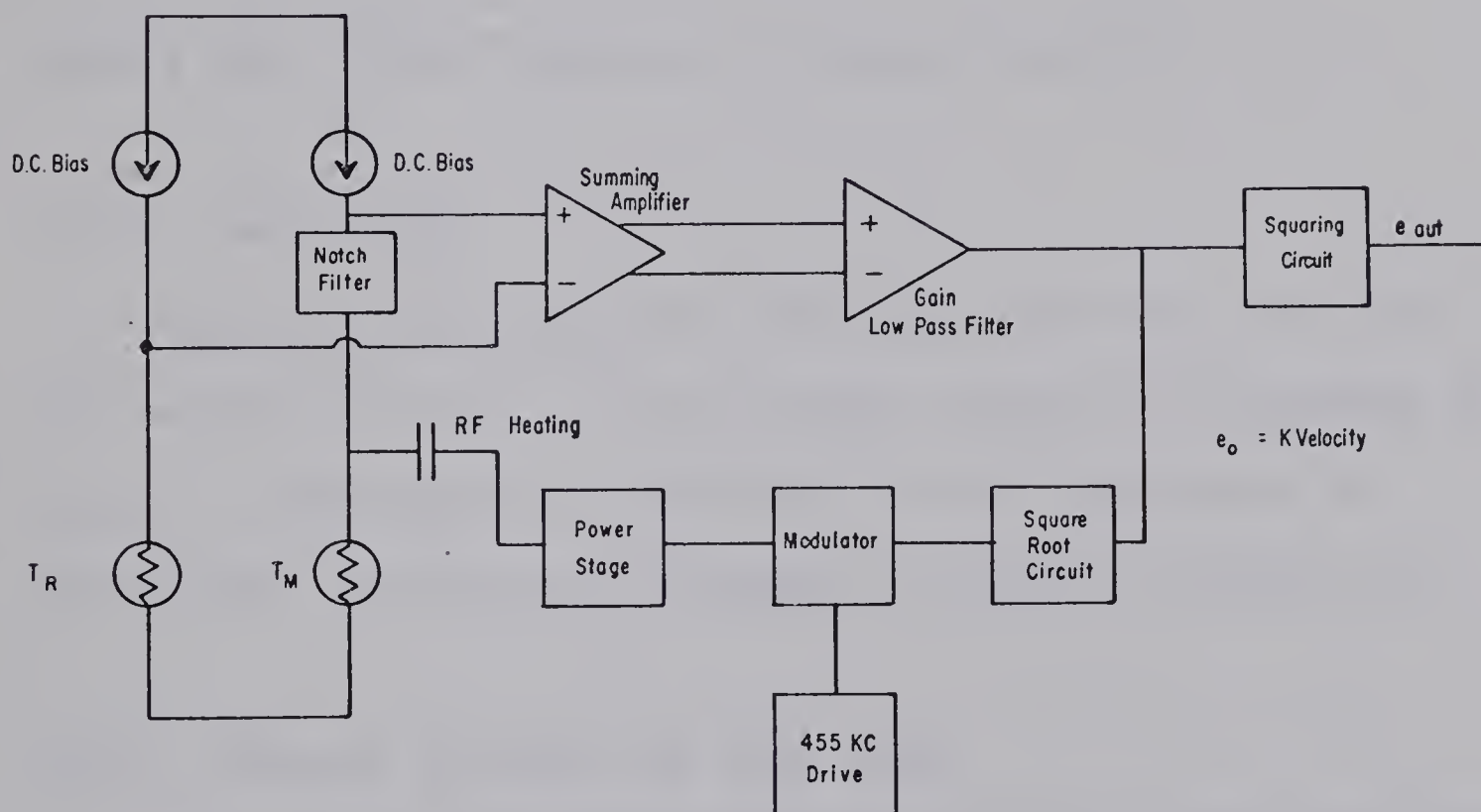


Figure A-1. Block diagram of flow meter.

## A2.2 Heating Circuit

The heating circuit which is the 455 KHz drive, the modulator and the power stage shown in Figure A-1 is shown in block diagram form in Figure A-2. The heating circuit



starts with a 910 KHz sine wave drive and the resulting RF power is a 455 KHz sine wave.

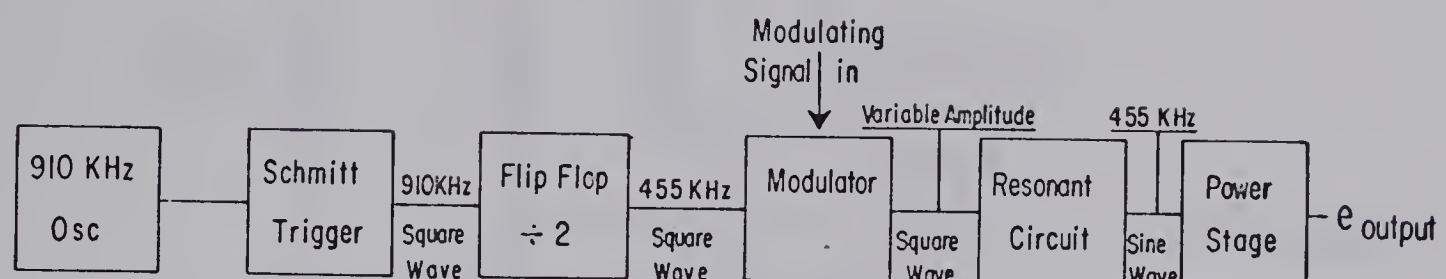


Figure A-2. Block diagram of heating circuit.

#### A2.2a Oscillator

The oscillator provides the basic 910 KHz drive for the heating circuit. A slug tuner allows the frequency to be set. The frequency stability of the oscillator is quite good. The circuit diagram is shown in Figure A-3.

#### A2.2b Schmitt Trigger and Flip Flop

The output from the oscillator drives a Fairchild 914 integrated circuit connected as a Schmitt trigger. This results in a 910 KHz square wave which varies from 0 to +3.6 volts. The output from the Schmitt drives a binary scaler. This binary scaler is a Fairchild 923 integrated circuit used as a counting flip flop. The output is a 1.5 v 455 KHz square wave.





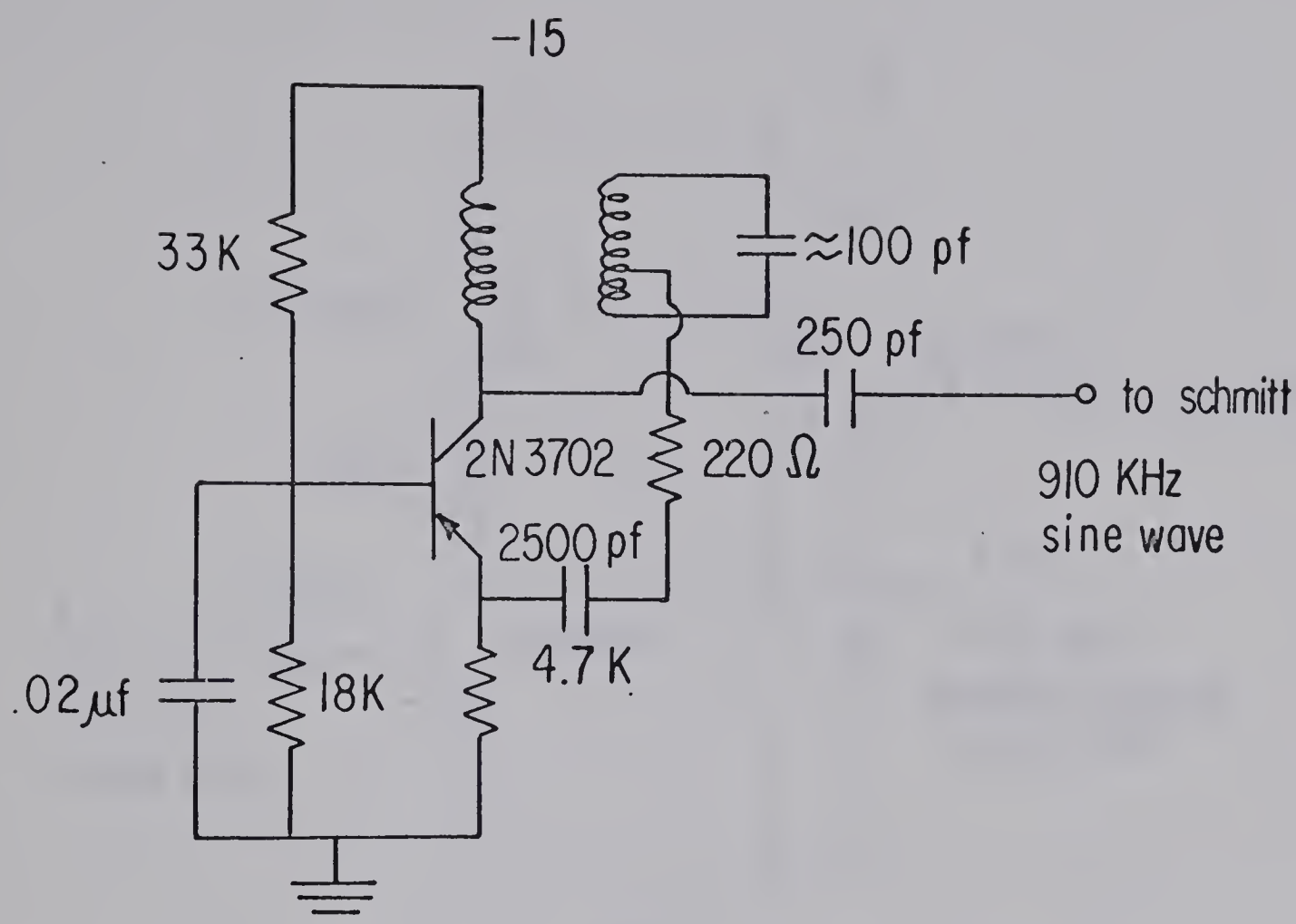


Figure A-3. Oscillator.

#### A2.2c Modulator

The output from the flip flop drives a switching transistor in and out of saturation. The square wave amplitude of the collector output is controlled by the variation of the supply voltage to the switching transistor. This is done by the modulating signal via the two emitter followers. This technique gives very linear amplitude modulation. The circuit diagram of the modulator is shown in Figure A-4.



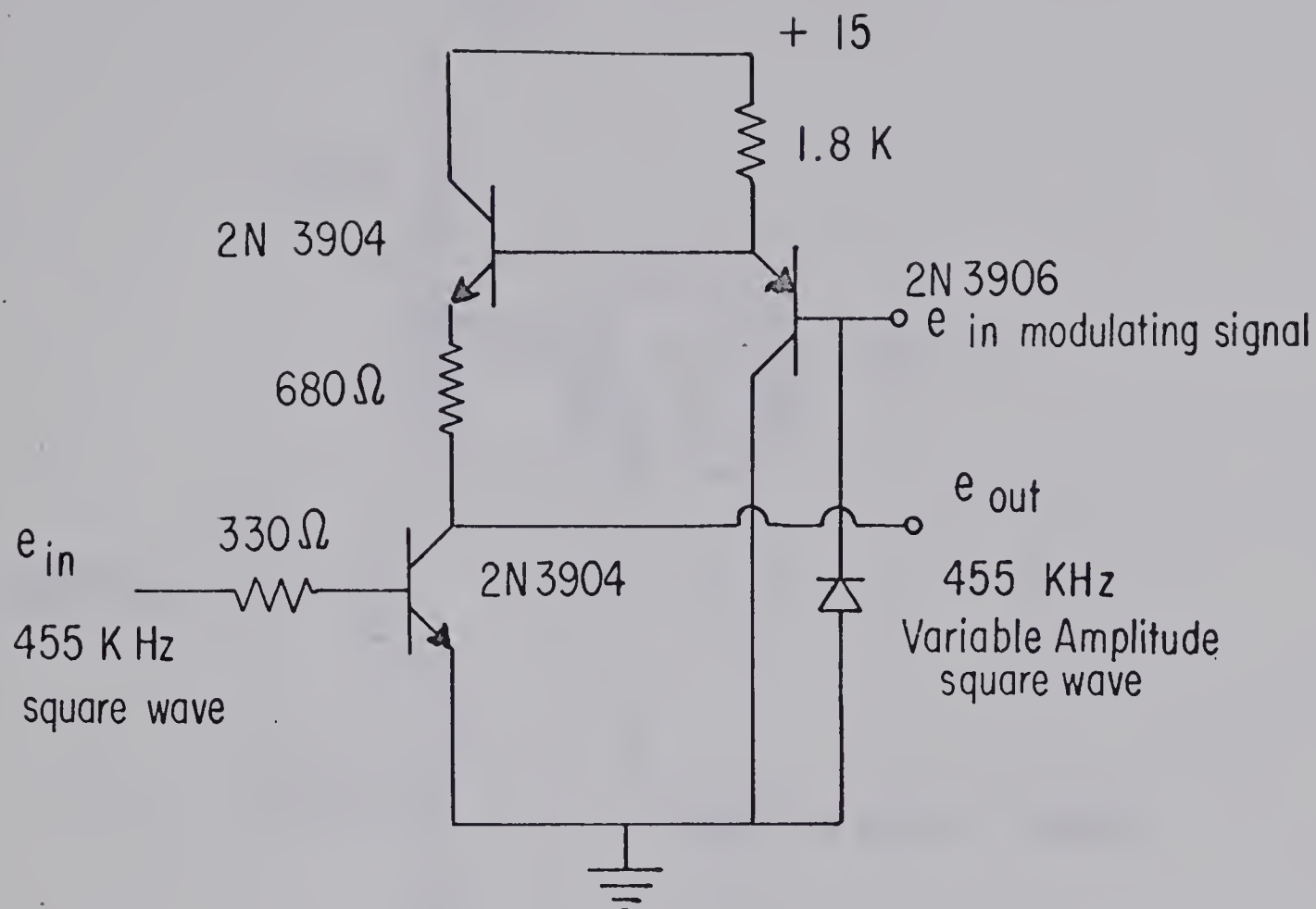


Figure A-4. Modulator.

#### A2.2d Resonant Amplifier

This amplifier converts the 455 KHz amplitude modulated square wave into a 455 KHz amplitude modulated sine wave. This is done by filtering out the harmonics so as to simplify the filtering procedure in the detecting system. This filtering can be done by using another resonant filter. The resistor across the coil is adjusted to load it in order to prevent it from over-driving the power stage. The circuit diagram of the resonant amplifier is shown in Figure A-5.



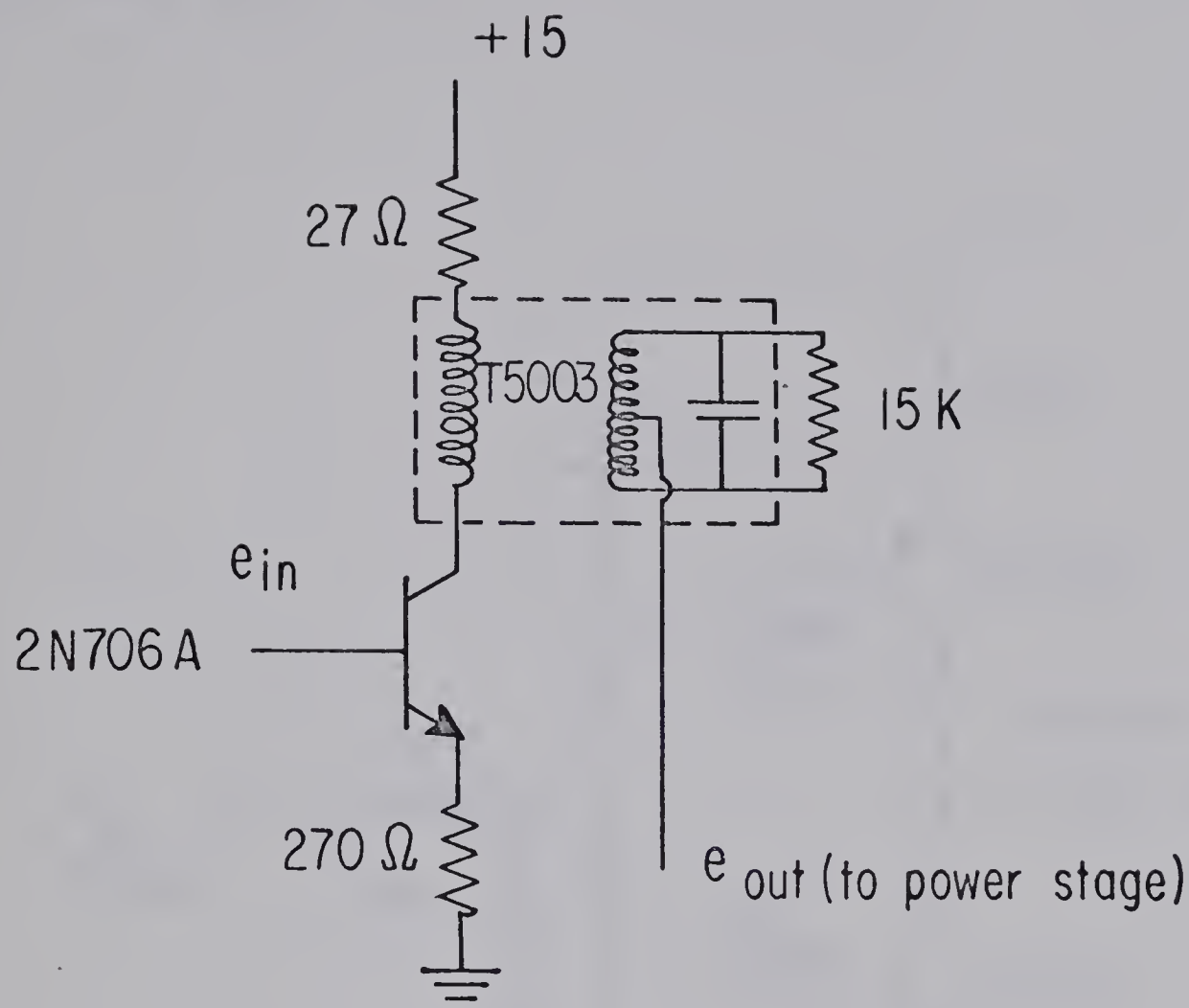


Figure A-5. Resonant amplifier.

#### A2.2e Power Stage

This stage provides the power to heat the measuring thermistor. It has the capability to provide almost  $\frac{1}{2}$  watt at 455 KHz. At zero flow it provides about 8 mw. to heat the thermistor above the ambient temperature of the blood. The RF power is coupled to the thermistor through a capacitor. The diodes bias the transistors so that they are turned on when the input is zero. This prevents cross-over distortion. The small resistors in series with the collectors and bases of the power transistors protect the



transistors against overload. The circuit diagram is shown in Figure A-6.

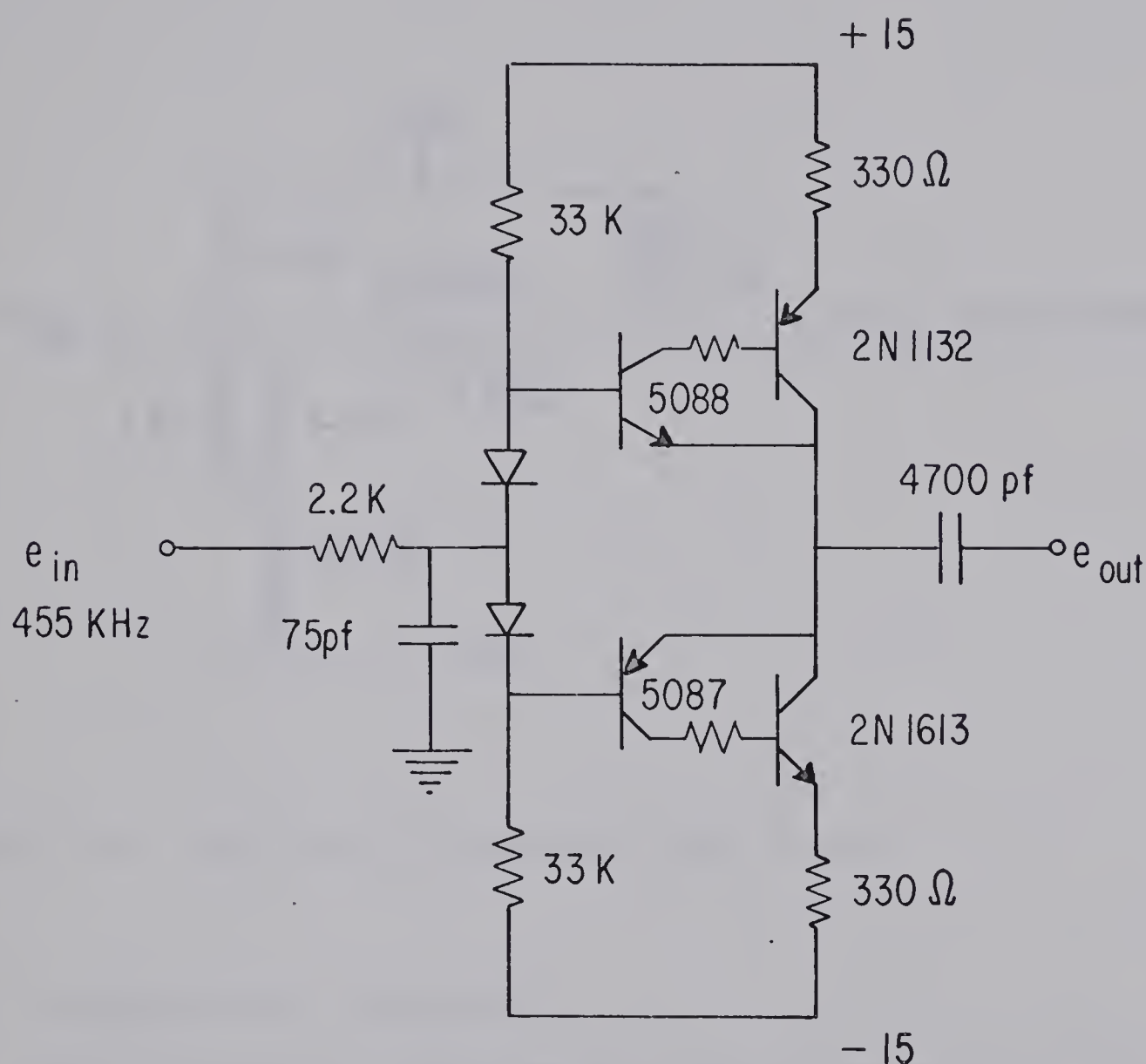


Figure A-6. Power stage.

### A2.3 Measuring Thermistor and Filter

The measuring thermistor was biased with a D.C. current of about 100 ua. This current through the thermistor resulted in a small voltage across the thermistor and hence changes in the resistance of the thermistor due to cooling were converted to voltage changes. The heating power was





filtered by the LC filter at the input of the amplifier. Additional filtering was provided by the 0.02 uf capacitor. The circuit diagram of the scheme is shown in Figure A-7.

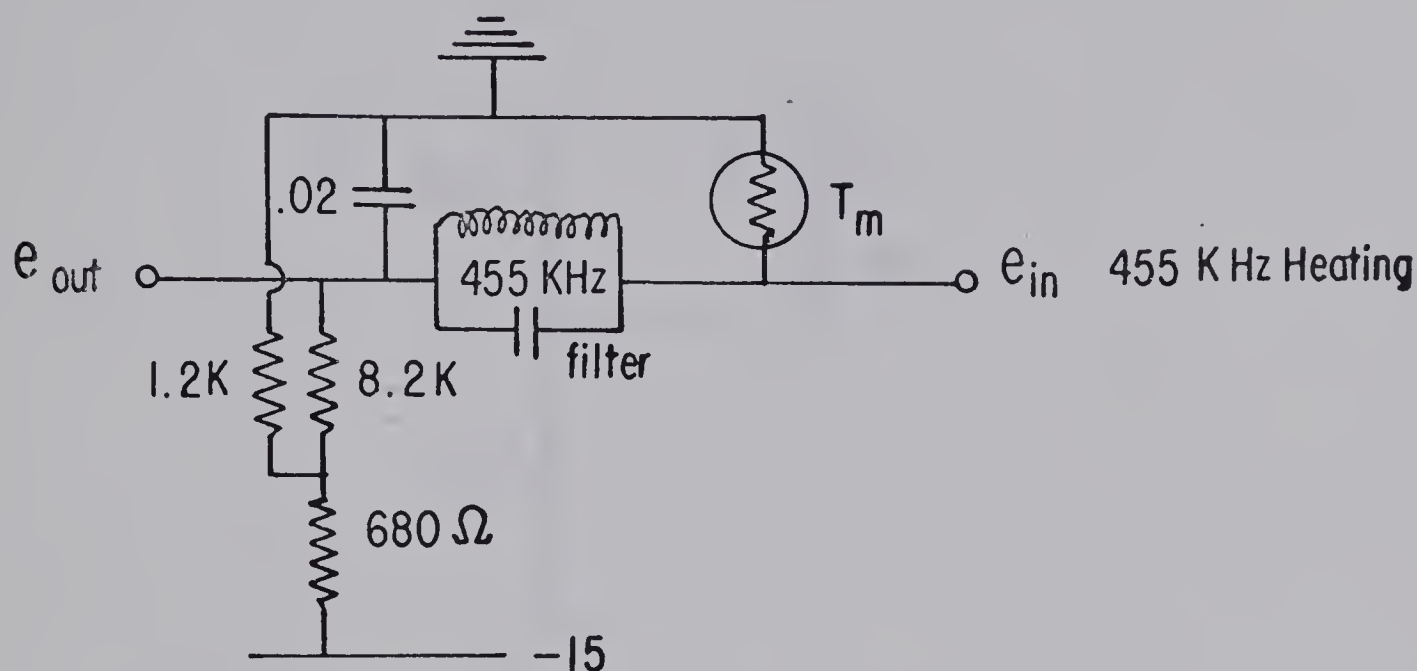


Figure A-7. Measuring thermistor and filter.

#### A2.4 Compensating Thermistor

This thermistor provides the zero flow balance for the system and also the compensation for temperature changes of the blood. The D.C. bias is about 30 - 50 uamps so that the voltage drop across the heated and reference thermistor is the same at zero flow. When the flow is not zero, the voltage across the reference thermistor does not change since it is insensitive to flow. A resistor (in the probe) balances the two temperature coefficients of resistance. The capacitor filters any heating power that may be present



in the thermistor due to coupling through the fluid. A circuit diagram is shown in Figure A-8.

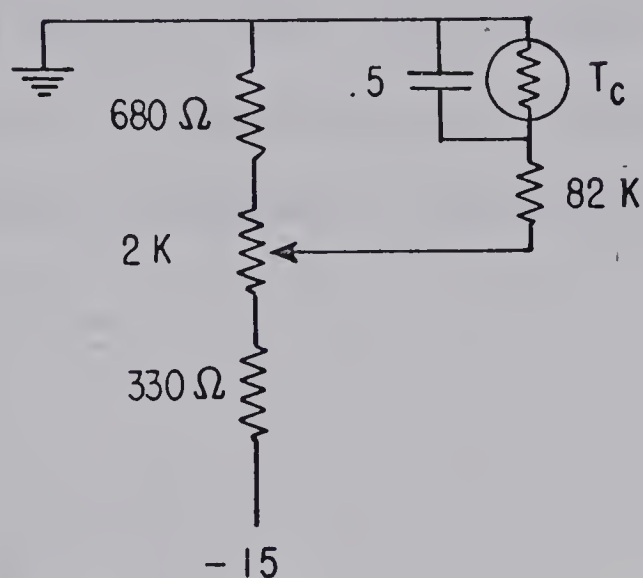


Figure A-8. Compensating thermistor.

#### A2.5 Amplifier

Since 455 KHz loads integrated circuit operational amplifiers, a discrete component amplifier was used as the input stage. The output amplifier was an integrated circuit operational amplifier. The voltage gain of this combination was  $10^4$ . The input stage was a temperature controlled differential pair of closely matched transistors. The drift of this combination was about 1 uV/degree C. The input stage was operated at a reduced supply voltage to improve noise performance. The operating current set



by the temperature compensated current source was set at 90 uamps for optimal noise performance and current gain. Protection diodes were also included in the input stage.

The input resistances of the second stage were adjusted so that the loading on the first stage was balanced. The feedback resistor of the second stage was split to give a gain of 100. This splitting was necessary so that a smaller feedback resistor could be used. Figure A-9 shows a circuit diagram of the amplifier circuit.

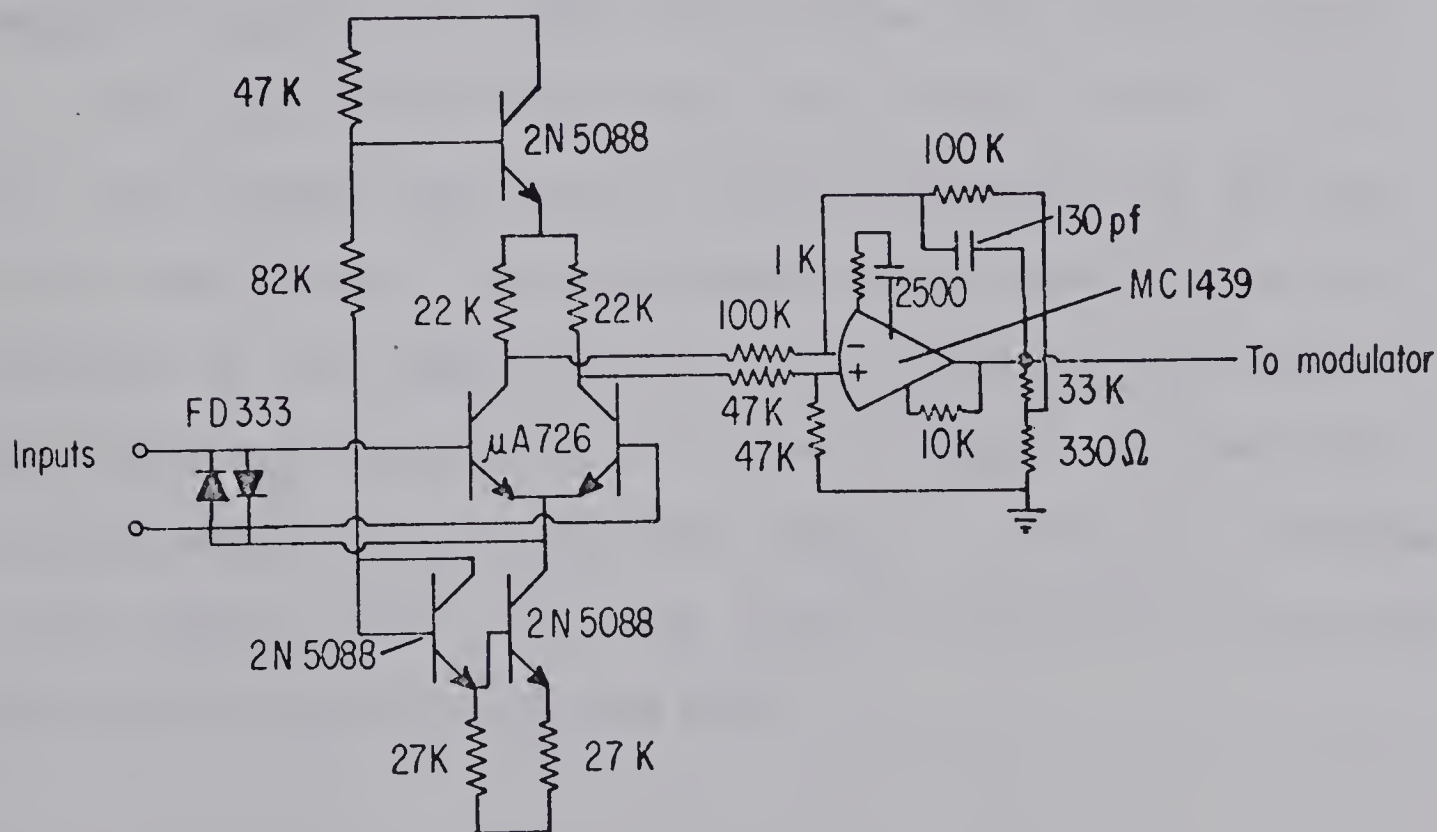


Figure A-9. Amplifier.





## A2.6 Linearizing Circuits

### A2.6a Square Root Circuit

This circuit was placed in the feedback path to compensate for the non-linearity due to the second power of current in Joule's Law. This circuit consists of two operational amplifiers; the first generating the non-linearity while the second provides the bias which heats the measuring thermistor above the ambient temperature of the blood. The second amplifier gain is less than one so that the non-linear amplifier can operate over a 10 volt range. It incorporates a compensator. The non-linearity is generated by turning the diodes on and decreasing the feedback resistance. The first diode turns on at about 0.5 volts and the second turns on at about 1 volt. All the other diodes turn on at 1 volt intervals set by the 33 ohm resistors. The non-linearity is generated so that 10 volts at the input results in 10 volts at the output. The 240 K and 360 K resistors were adjusted to give the correct value of gain and power bias. A schematic diagram of the square root circuit is shown in Figure A-10 and of the diode network in Figure A-11.

### A2.6b Squaring Circuit

This circuit provides compensation for the non-linearity due to the heat transfer from the thermistor. The first amplifier provides a variable gain through a range switch to provide variable sensitivities and allow



the output to operate over the major portion of the generated non-linearity. The range switch varies the flow sensitivities from 1 to a sensitivity 100 times greater. The squaring amplifier operates similarly to the square root circuit except that the diode network is in series with the input. The first amplifier has a corner frequency of 160 Hz while the second amplifier has a corner frequency of 190 Hz. The non-linearity was set so that 10 volts at the input resulted in a 10 volt output. This was set by making a fine adjustment in the gain of the first amplifier (50 ml./min. = 10 V out). Figure A-12 shows a circuit diagram of the squaring circuit and the range control. Figure A-13 shows a circuit diagram of the diode network.

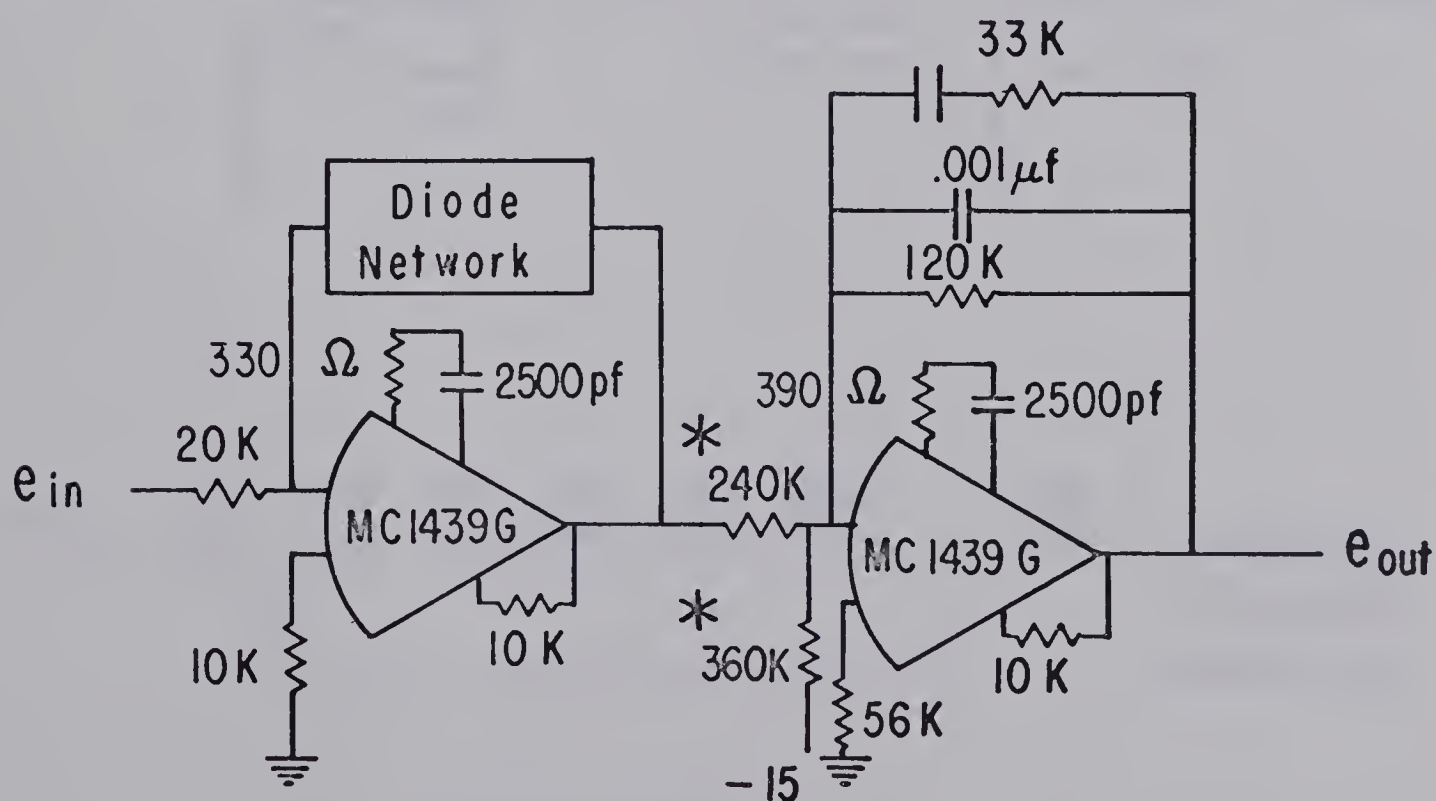


Figure A-10. Square root circuit.



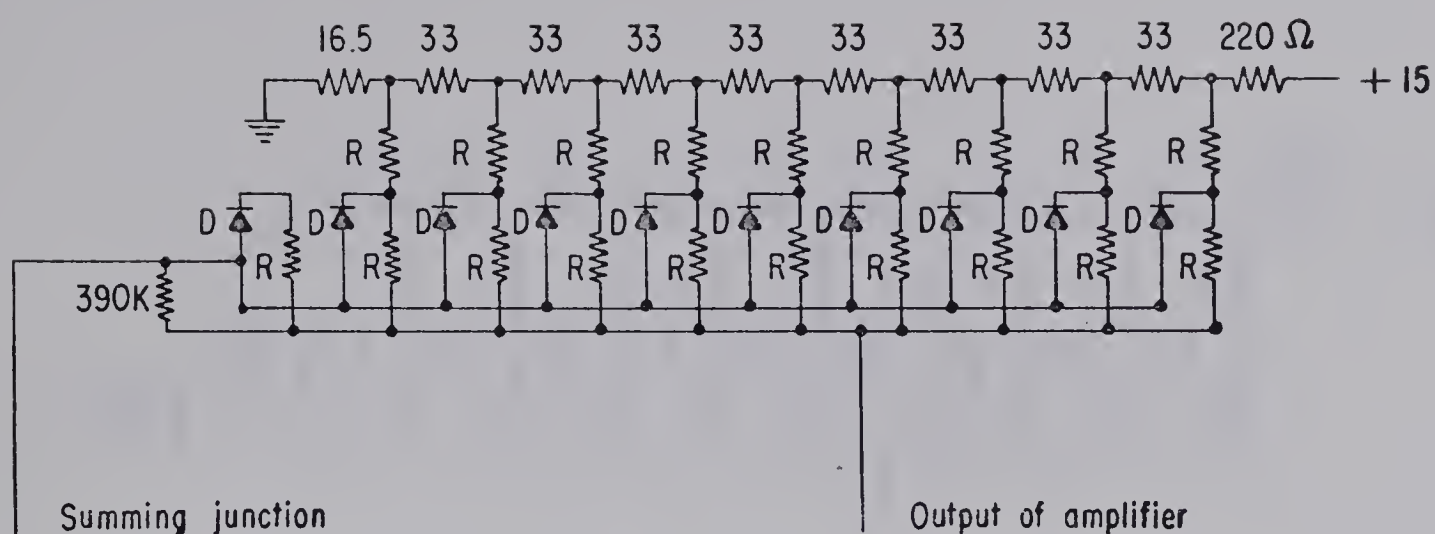


Figure A-11. Diode network.

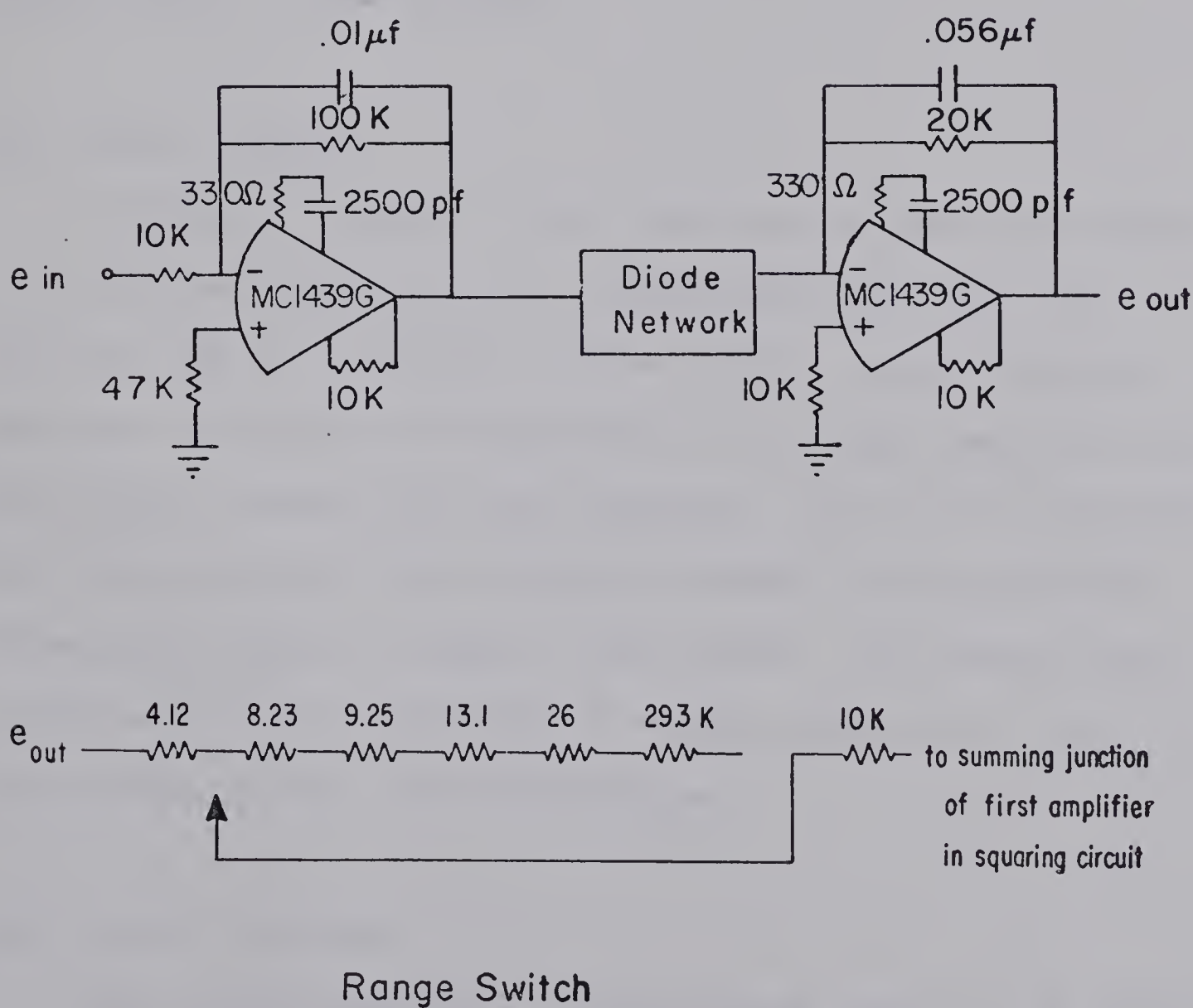


Figure A-12. Squaring circuit and range control.



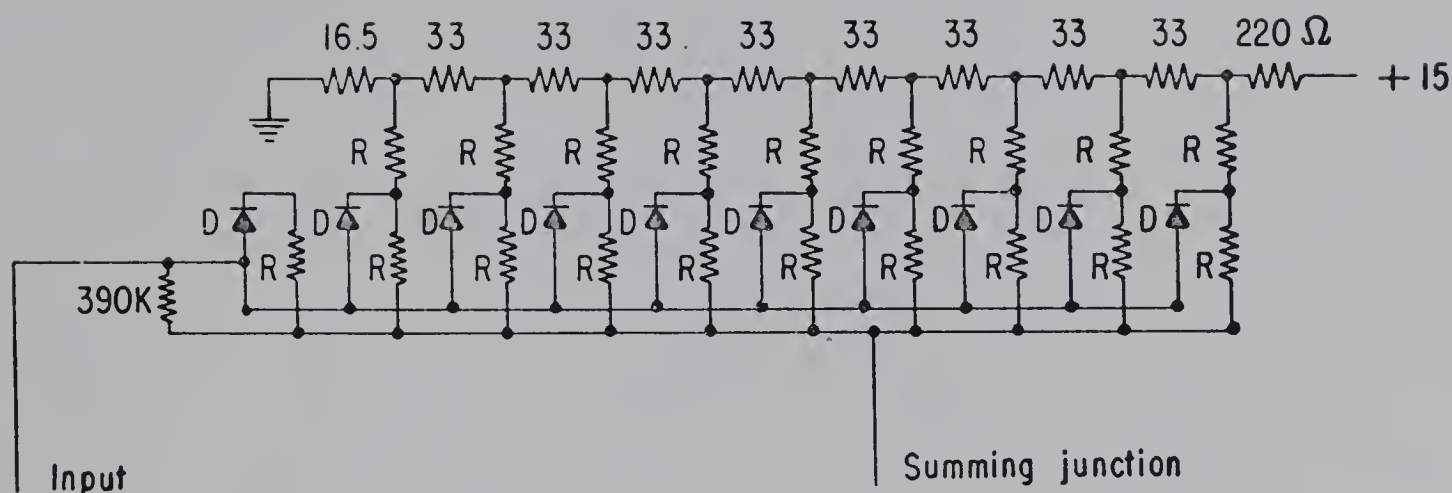


Figure A-13. Diode network.

#### A2.7 Meter Circuit

A meter (50 ma 0 - 50 ma) was used in the flow meter. It serves two functions; as a balancing meter for zero velocity and as a readout of flow rate. In the balance position the meter is connected to the output amplifier of the system (before the range switch). In the flow position the meter measures the linearized output voltage of the system. The sensitivity of the meter is reduced with a series resistor so that 10 volts gives a full scale deflection.

#### A2.8 Power Supplies

The system requires  $\pm 15$  volt power supplies at 200 ma. Regulated power supplies were designed to provide  $\pm 15$  volts at 500 ma.







**B29983**